COST final meeting
March 20-21 2018
Leuven
Abstract book
Scientific committee

Sebastien Carpentier
Rick Van de Zedde
Astrid Junker
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Local organizing committee

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Abstract book editing

Sebastien Carpentier, Nadia Campos

Website & registration system.

Ulrich Stegelmann
Practical information

Bus center (station) to campus:

Bus number 2 direction Heverlee Campus from platform 6, every 15 min. Get out at Kantineplein.
Bus number 1 direction Heverlee Campus from platform 6, every hour. Get out at Kantineplein.
Bus number 616 direction Zaventem from platform 14, every hour. Get out at Kantineplein.

Conference dinner

For those that registered for the conference dinner.

Tuesday 20 March 2018 at 19.30h.

SALONS GEORGES
Hogeschoolplein 15
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Program

March 20, 2018

08:30-09:30 Registration/good morning coffee

09:30-09:35 Welcome by the COST chair Sebastien Carpentier

Phenotyping Crop Biodiversity – chair: E Rosenqvist (DK)

Invited speaker
09:35-10:15

Kristian Thorup University of Copenhagen, (DK). “Semi-field phenotyping for root growth and function, early results from the RadiMax root phenotyping facility”

Invited speaker
10:15-10:55

Benjamin Kilian CROP TRUST, (GE) “Reaching back through the domestication bottleneck to feed a hot and crowded planet”

10:55-11:20 Coffee Break

- Orals (3x20 min)

11:20-11:40 S Carpentier (BE) The quest for climate smart varieties: phenotyping the banana biodiversity present in the gene bank

11:40-12:00 A Junker (DE) High-throughput quantitative analysis of shoot properties in diverse maize Genebank accessions

12:00-12:20 C Ottosen (DK) Heat priming effects on anthesis heat stress in wheat cultivars with contrasting tolerance to heat stress

12:20-14:00 Lunch- Break Poster Session

14:00-15:00 Visit of the KULeuven/Bioversity International gene bank and phenotyping facilities
• Orals (2x20 min)
15:00-15:20 P Mylona (GR) Phenotyping legume crop performance: bottlenecks and solutions
15:20-15:40 M Maia (PT) Grapevine resistance to Plasmopara viticola: combining metabolomics and phenotyping for biomarker discovery
15:40-16:20 Coffee Break Poster Session

Integrated Solutions for high-end phenotyping – chair: X Draye (BE)

• Orals (3x20 min)
16:20-16:40 U Schurr (DE) Phenotyping from lab to field – technologies, concepts and integration
16:40-17:00 M Neal (UK) Low-cost sensors for environmental and crop growth monitoring in agricultural settings.
17:00-17:20 J Westergaard (DK) "PlotCut 2: Developing a high-throughput UAS (drone) image-data extraction tool for plant breeders with user-friendliness and business needs as the main priority."

Invited speaker

17:20-18:00 Cyril Pommier INRA; (FR), "Standardization in the phenotyping community."

18:30-19:30 Guided tour in Leuven

19:30 Conference dinner
March 21, 2018

08:30-09:00 Registration

Integrated Solutions for high-end phenotyping – chair: X Draye (BE)

Invited speaker

09:00-09:40 Christophe Salon, INRA, (FR) In the context of sustainable agriculture how, with plant high throughput phenotyping, can we address the various challenges?

Orals (2x20 min)

09:40-10:00 D Raymaekers (BE) Drone based phenotyping for plant breeders

10:00-10:20 J Marques da Silva (PT) Using reflectance spectroscopy and artificial intelligence techniques to automatically identify maize genotypes and water stress physiological effects

10:20-11:00 Coffee Break

Phenotyping for future climate scenarios – chair: R van de Zedde (NL)

Orals (3x20 min)

11:00-11:20 P Peltonen-Sainio (FI) Diversity Induced Improvements in Resilience to Climate Change - The Scale Matters


11:40-12:00 I Rieu (NL) Reproductive thermotolerance in tomato - pollen are key

12:00-13:00 Lunch Break

13:00-13:30 Poster speed presentations

13:30-14:30 Lunch Break
Discussion  COST, what's next? EMHPASIS, COST outputs – chair: S Carpentier (BE) U Schurr (DE)

13:30-14:30  Live audio interrogation with online tool.

Phenotyping for future climate scenarios

- Invited speaker

14:30-15:10  Edith Lammerts van Bueren, WUR (NL), "Perspectives of breeding for ecological resilience"

15:10-15:40  Coffee Break

- Orals (2x20 min)

15:40-16:00  M Kuska (DE) Combined analysis of gene expression and optical reflectance properties of barley differing in their resistances to powdery mildew

16:00-16:20  A Virosta (ES) Impact of water deficit on growth and yield components of two garlic (Allium sativum L.) cultivars at different crop stages

16:20-17:00  Invited speaker Arno Ruckelhausen (DE) Technological tools for field-based phenotyping – about multi imaging sensor data fusion and (autonomous) platforms

18:00-19:00  MC/ Core meeting: Wrap up FA1306, reporting and what’s next.
We have recently built a facility allowing root phenotyping of 600 plant lines to 2.8m depth using minirhizotrons. In this and other semifield setups direct root phenotyping have been compared to indirect phenotyping of root activity based on shoot measurements of tracer uptake or water stress.
Crop wild relatives (CWR) represent a large pool of genetic diversity from which to draw beneficial allelic variation required in breeding programs. CWR have been extremely valuable in adapting crop varieties to changing disease pressures, farming practices, market demands, and climatic conditions. This talk will provide examples on how wild species have contributed to the development of improved crop varieties and where efforts must be concentrated in order to harness their value in the future. Introducing the global initiative “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives” and drawing on it’s current results, the role that CWR play in modern crop breeding will be documented. Activities that promise to facilitate the use of CWR, and what constraints continue to hinder increased utilization of plant genetic resources in breeding, will be discussed.
Plant Phenotyping data management following the FAIR principles (Findable, Accessible, Interoperable, Reusable) is highly challenging because of its heterogeneity. Thus, simply integrating and consolidating data within a single dataset like a phenotyping network is already a complicated task which is even more complex when trying to link different datasets together for long term data reuse. The Minimal Information About Plant Phenotyping Experiment (MIAPPE) data standard have been built to answer this challenge. It is s included in several systems including production databases like PHIS, data repositories like GnpIS or eDale, file formats like ISA Tab, and in a web service, the Breeding API. This enables interoperability in a growing list of tools and databases. We will review those implementations and their benefits, show its current adoption state and detail the future plans for phenotyping data standard.
In the context of sustainable agriculture how, with plant high throughput phenotyping, can we address the various challenges?

Christophe Salon¹,², Céline Bernard¹,², Philippe Declerk³, Daniel Just⁴, Marion Prudent¹ and Christophe Rothan⁴, Christian Jeudy¹,².

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In a context of climate change and soil and water resource degradation, it becomes increasingly important to reduce the need for high nutrient, water, or pesticides inputs, leading to more sustainable agricultural practices. In this context, our aim is to select the best performing crops in various deleterious abiotic environments, having both a higher yield and a better quality, a better environmental « efficiency ». Because plants interact with numerous and diverse microorganisms, especially in the soil volume surrounding roots, called rhizosphere, plant-microorganism relationships in the rhizosphere is also of great agronomical and ecological importance. To address these challenges, high-throughput, non-invasive systems/methods have been developed to characterize the numerous phenotypic traits involved. Associated methods will be shortly described. Examples arising from various ongoing projects will demonstrate how shoot, root or fruit phenotyping can be applied to various thematic and crops to identify either elite genotypes face to a given abiotic stress (e.g. iron chlorosis, drought stress or nutrient deficiency), follow fruit development or identify plant and microorganism beneficial interactions.
Session: **Phenotyping for future climate scenarios**

**Invited speaker**

**5 - Perspectives of breeding for ecological resilience**

*Edith Lammerts van Bueren, WUR*

*Presenting author: Edith Lammerts van Bueren*  
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Breeding robust varieties for a farming system, such as organic agriculture, optimizing ecological resilience and relying on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects, is a great challenge. Such farming systems require multifunctional and flexible varieties.  

i) Replacing water soluble mineral fertilizers, that can readily be taken up by the plant, by lower inputs of organic, slow releasing fertilizers requires varieties with an explorative and flexible root systems, early vigor and nutrient efficiency.  

ii) Refraining from chemical-synthetic herbicides requires crop varieties with good weed suppressive ability through various (combination of) traits such as plant length, leaf width and length, early vigor or early planophyl rather than erectophyl growth habit.  

iii) Reducing or refraining from chemical-synthetic fungicides and pesticides demands varieties with a high level of resistance or tolerance. This can sometimes be achieved by morphological traits such as waxy leaves in cabbage against thrips infestation, or plant length allowing the ears of cereals to ripen above the moisture leaf area and be less prone to ear diseases.  

iv) The organic sector is also exploring options to apply above-soil-variation within and between crop species to enhance buffering capacity against unpredictable and harsh weather patterns such as composite cross populations in cereals or crop mixtures such as wheat-legume combinations.  

v) Even more challenging is breeding for varieties with enhanced below-ground traits benefitting from plant-soil microbe interactions. However, sustainable farming systems not only focus on short-term benefits of yield stability but also demand focus on breeding for long-term perspectives of ecosystems services such as including flower traits that benefit to feeding pollinators or enhancing root biomass to increase soil organic matter for soil conservation, water provision and carbon sequestration. Such aspects are complex traits and currently limited to breeding research and not yet taken up by commercial breeding as it also requires adaptation of selection strategies.
For field based phenotyping robust data acquisition with high spatial and temporal resolution is of high relevance, thus typically image based sensor systems are in focus. Moreover, due to demands for detecting different crop characteristics under complex and varying field as well as environmental conditions sensor data fusion is a promising approach. In order to combine data from different sensors with varying frame rates, data volumes and geometric positions a reliable data acquisition system is required. The concept BreedVision allows the data acquisition of various sensors – such as hyperspectral or light-curtain imaging – and analysis down to the level of an individual plant. A self-propelled mobile platform with automatic height adjustment of the sensor module and flexibility with respect to crop height and row width can perform field measurements with up to 2000 plots per day. Data analysis can be applied for several crop parameters, as for example for non-destructive biomass detection. For smaller crops the autonomous field robot platform BoniRob with corresponding “Phenotyping Apps” has the potential for autonomous field phenotyping. First results of field experiments with a multi-wavelength laser line profile system attached to BoniRob – using spectral features and 3D surfaces in a single sensor system - demonstrate the potential of both, platforms and imaging systems.
Maize (Zea mays L.) genetic resources must be explored to foster plant breeding, required to cope with emerging abiotic stresses, namely the increased drought episodes posed by global climate change. The development of methodologies able to deliver a quick and accurate identification of plant genotypes and to correctly assess plant physiological status will be useful for both plant breeding and precision agriculture. Diagnostic assays based on optical techniques have the advantage of being non-invasive and time- and cost-effective, being therefore operative in high-throughput plant phenotyping. In this work, a combination of reflectance spectroscopy and machine learning techniques was used to develop a non-invasive method for the automatic identification of maize genotypes and for the assessment of plant water stress. Reflectance spectra were obtained from intact leaves of well-watered plants of the maize cultivars Pioneer P0023 (drought resistant) and Pioneer P9838 (drought sensitive), as well as from moderately and severely water-stressed plants from both cultivars. Collected data was used to construct classifiers based on decision trees and neural networks. Results show that decision trees applied to reflectance spectroscopy data could distinguish between the maize genotypes, with classification success reaching almost 100%. This procedure was also able to automatically distinguish between the physiological status of control, moderately water-stressed and severely water stressed plants, reaching almost 100% success in Pioneer 9838 and slightly less (95%) in Pioneer P0023. Preliminary application of neural networks to spectral reflectance data did not improve the classification success, but this work is still in progress. The results suggest that the conjugation of reflectance spectroscopy with artificial intelligence techniques may contribute to accelerate the breeding of improved drought-resistant maize genotypes.
8 - Low-cost sensors for environmental and crop growth monitoring in agricultural settings.

Dr. Mark Neal¹, Dr. Tom Blanchard²

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Phenotyping is now well-established, and there is a wide range of technologies and off-the-shelf systems that can assess the performance of crop varieties grown under glass and in well-constrained environments. Many of these systems and technologies are expensive and depend upon regular maintenance and protection from the environment and theft. We have been working on low-cost sensors for deployment in on-farm settings and with very low maintenance regimes. I will present two low-cost (£200 - £300) sensor systems suitable for deployment in conventional agricultural settings and data that has been captured during trials and deployments. The first is a wireless micro-climate monitor and the second is a crop growth sensor. A network of the micro-climate monitors has been deployed in maize on a model farm in Zambia (for an 18 month period with zero maintenance) and in field margins in the U.K. also for extended periods. The crop growth sensor is currently undergoing final laboratory trials and is due for deployment in the field on a barley crop in the spring. The micro-climate monitor includes temperature, humidity and light intensity sensors and can also be equipped with a soil moisture sensor. The crop growth sensor uses sonar and infra-red transducers to measure crop height and to provide indications of the density, and potentially an estimate of biomass. Trials for the sensors are on-going and Ystumtec Ltd. and Aberystwyth University would welcome collaboration on their testing and future development.
Drone based, or drone assisted phenotyping has been described in the literature for many crops and traits with generally high accuracies in comparison to field based methods. Homan et al (2016) found for example consistently low RMSE values (0.07, 0.02 and 0.03 m for May, June and July, respectively) for drone based wheat height measurements, enabling crop growth rate to be derived from multi-temporal surface models. Also the drone industry has pointed towards agriculture as one of the leading industries for drone use. However, the practical implementation into the experimental fields of plant breeders or public research facilities has been limited to demonstrations and publicly funded research programs. The step towards fully exploiting the potential of drone based phenotyping requires (i) the simplification of data acquisition and data handling, (ii) data analytics which are focussed to extract relevant agrometrics on micro-plot level and (iii) the integration within day-to-day workflows. Over the past 2 years, we have collaborated with plant breeders and public research facilities to develop a drone image processing workflow dedicated to experimental fields. Plant height for wheat and corn plots, diseases in sugar beets and wheat trials, plant emergence for potatoes and spinach are just a few traits for which image analytics were provided and which resulted in agrometrics which reaches or even surpassed field based measurements.
Plant phenotyping is an essential tool for many applications ranging from functional genomics, to (pre-)breeding, breeding and analysis of biodiversity. It develops rapidly into a bottleneck for progress in basic and applied research. Lack of adequate solutions for quantitative analysis of plant architecture and function as well as their interaction with the dynamic and heterogeneous environment hampers progress in basic sciences as well as in breeding-related research. In recent years, significant interdisciplinary approaches have been started to overcome this “phenotyping bottleneck”. Techniques were developed to quantify the dynamics and the heterogeneity of plant structure and function as well as of environmental cues. In this presentation we will explain recent results from the phenotyping chain approach, by which we study the relevance of phenotyping technologies at various scales from the lab to the field in direct experimental approaches and from meta-analysis. The integration of different scales is also a central element of EMPHASIS: the pan-European for Multi-Site Plant Phenotyping And Simulation for Food Security in a Changing Climate, which is developing on the basis of the portfolio of existing national plant phenotyping centers in Europe. Here we will discuss the recent developments since EMPHASIS has been established as a ESFRI project.
11 - PlotCut 2: Developing a high-throughput UAS (drone) image-data extraction tool for plant breeders with user-friendliness and business needs as the main priority.

Jesper Cairo.
Westergaard, University of Copenhagen.
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At the University of Copenhagen, our unmanned aerial systems (UAS, drones) image research fields with hundreds of breeding plots using RGB + multispectral + thermal sensors several times a week. Together with Nordic plant breeders (in the 6P project) we have also been imaging and handling their data extraction from fields with several thousand plots. To do this we developed PlotCut (version 1), a prototype. It did the job, but it was not user-friendly, so it was time to develop something the breeders themselves could and would use, so that their UAS-derived data could become a part of the decision support systems in their plant breeding.

Enter PlotCut 2. A stand-alone program created for the breeders. The main demand for version 2 was ease of use. It should be as intuitive as possible. In order to develop software like this, the biggest part has actually been the user-interface testing. We try to think as much as possible as the end-user in the initial development and then we have the breeders themselves test the software. To put it mildly, it takes a huge amount of extra time with all the development iterations, but it pays off with software that doesn’t suit the programmer’s good but sometimes misguided intentions but instead is tailored to the users’ - the plant breeders’ – actual needs.

PlotCut will keep being developed as a stand-alone product to ensure a production-ready high-throughput software for the breeders and ourselves. At the same time, we are contemplating a QGIS based version where everything is georeferenced, where we can include soil profile data, landscapes gradients, climate data, historical data and many other information carrying layers. But here we will loosen the otherwise strict demands for user-friendliness and instead go for maximum features. Developing along these two parallel tracks, means we can avoid the bottleneck we used to have regarding data extraction, but at the same time try out our more advanced, even esoteric, ideas.
Bananas are a very important crop for the African highlands. The region faces water deficit and low growing degree days. Bioversity International holds the world’s biggest collection of banana biodiversity (>1500 accessions). We aim to evaluate the potential of our collection via phenotyping.

We have characterized the growth potential of 32 representatives of the Musa biodiversity and have ranked their growth under control and mild osmotic stress conditions. 5 representatives, representing 51 related accessions in the collection, were found to have superior growth. This growth models are being scaled up to a growtainer. Our growtainer simulates the climate of the highlands and grows 504 plants simultaneously.

To get more insight into the physiology, we monitor in a next step the individual plant transpiration of selected genotypes gravimetrically. We gained already insight in the day to day, and within day, variations of transpiration rate, of all 32 representatives under controlled conditions. We show that banana declines its transpiration rate near the end of the day, reducing transpiration water loss up to 30%. This feature is correlated to efficient water consumption and is thus a valuable, rapid screening parameter for drought tolerance. We hypothesize that this might be a feedback from photosynthesis.

With sufficient root pressure and less need to fix CO2, increase the stomatal resistance for gas exchange results in a higher water use efficiency. We have selected 25 phenotypic variables related to the transpiration pattern. Based on blind clustering within the 25 dimensional space we detect two major feedback phenotypes in the Musa biodiversity: water consuming cultivars with a late feedback, and cultivars with a balanced water usage. Breaking up, and describing the daily transpiration patterns proves useful as a cultivar selection tool, while also allowing insight in the plant physiology of transpiration.
High-throughput quantitative analysis of shoot properties in diverse maize Genebank accessions

Astrid Junker, IPK Gatersleben, Henning Tschiersch, IPK Gatersleben, Beatrice Knüpfer, IPK Gatersleben, Andrea Bräutigam, University of Bielefeld, Martin Mascher, IPK Gatersleben, Michael Grau, IPK Gatersleben, Andreas Börner, IPK Gatersleben, Thomas Altmann, IPK Gatersleben

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The Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany, houses a comprehensive high through put plant phenotyping infrastructure for the quantitative assessment of the dynamics of growth related and physiological parameters in model and crop plants. Using these installations we are performing a large-scale phenotypic and genotypic characterization of the complete IPK Genebank maize collection comprising about 1300 accessions (enriched for Eastern European material). This study aims at the identification of accessions with interesting and agronomically relevant features and the elucidation of the molecular-genetic basis of trait expression. Experiments with two different temperature regimes (constant warm or gradually increasing) have been performed with successive subsets of lines, including a reference panel and a set of commercial maize hybrids for benchmarking purposes. We were able to identify Genebank inbred accessions which are comparable to commercial hybrids with respect to biomass formation under both temperature regimes as well as accessions with low sensitivity to cold temperatures. Additional measurements of the variation in photosystem II (PSII) performance (Tschiersch et al. 2017) revealed remarkably contrasting PSII efficiency values between hybrids and Genebank accessions (inbreds) in the early seedling stage. Moreover, our experimental approach also included investigations about the potential influence of energy allocation between photochemical and non-photochemical processes during light adaptation on growth performance. The assessment of plant traits that underlie the biomass component of the yield equation, namely factors influencing RUE (e.g. plant architecture) and light use efficiency (e.g. PSII operating efficiency) as well as allocation of absorbed light energy will enable to get a deeper understanding of the structural functional relationships for yield improvement.
Grapevine resistance to Plasmopara viticola: combining metabolomics and phenotyping for biomarker discovery

Marisa Maia\textsuperscript{1}, Rebecca Hofle\textsuperscript{2}, Ana R. Cavaco, Antonio Ferreira\textsuperscript{1}, Carlos Cordeiro\textsuperscript{1}, Reinhard Topfer\textsuperscript{2}, Anna Kicherer\textsuperscript{2}, Andreia Figueiredo\textsuperscript{3}, Marta, Sousa Silva\textsuperscript{1}

\textsuperscript{1} Lab FTICR e Espectrometria de Massa Estrutural; BioISI; CQB, \textsuperscript{2} JKI-Inst for Grapevine Breeding, \textsuperscript{3} BioISI.

Presenting author: Marisa Maia  

Grapevine (Vitis vinifera L.) is the most important fruit plant in the world with a major economic and medical importance. The domesticated V. vinifera cultivars frequently used for wine production are highly susceptible to fungal diseases, being downy mildew, caused by Plasmopara viticola, one of the most destructive vineyard diseases. Downy mildew affects all the green parts of the vine, causing yield reduction and significant production losses. Thus, if not controlled, it presents serious negative effects in several countries’ economy. To cope with this threat, the application of pesticides is the current strategy. This is not the most efficient and environmental friendly approach. The creation of new cultivars by breeding, as done by the JKI, one of the main institute for grapevine breeding in Europe, is the most sustainable approach. Resistance to P. viticola (RPV) in hybrid plants is achieved by crossing suitable parent lines or cultivars and the subsequent selection in the offspring to identify desired combinations of traits.

Currently mildew resistance in the hybrid lines is access by an infection-based leaf disc assay using the OIV descriptors to rate the resistance. This technique is time-consuming and laborious, thus at JKI researchers are using sensor based approaches (RGB, hyperspectral imaging) to complement this evaluation. Metabolomics is becoming one of the most widely used approaches to evaluate health related changes in crop plants. Combining hyperspectral imaging with metabolomics data would contribute to a better discrimination between resistant and susceptible cultivars. To achieve this goal, leaf disc inoculations were conducted in 8 different hybrids genotypes, with different RPV loci, to evaluate the degree of resistance (work developed within the STSM). A leaf disc time-course experiment was performed (0, 12, 24, 48, 72 and 96 hpi), hyperspectral imaging and metabolomics analysis is currently ongoing.
Phenotyping legume crop performance: bottlenecks and solutions
Photini Mylona, Greek Gene Bank, Institute of Plant Breeding & Genetic Resources, Greece and Alexios N. Polidoro, Department of Genetics and Plant Breeding, School of Agriculture, Aristotle University of Thessaloniki, Greece.
Presenting author: Photini Mylona phmylona@nagref.gr

Phenotyping of crop performance is an indispensable tool in plant breeding. The advent of technological methods, tools and platforms over the last years have allowed for a vast development of phenomics studies. However, the question of how this systems and tools could be utilized to provide an integrated and unified characterization of genotypes under various field conditions still remains a challenge. In that respect, a number of parameters and characteristics are often scored to provide a, as much as possible, detailed description of ideotypes closely associated to the quantifiable genetic traits. The challenges imposed by climate changes further highlight the need for concerted research approaches in order to develop crops that are able to cope with environmental stresses, while increasing yield and quality in rainfed cropping systems. Legume crops are subjected to biotic stresses that in association to abiotic stress may diminish crop yield and quality. The study is focused on evaluation of selected grain legume genotypes including winter pea, chickpea, faba bean, lupin and lathyrus. Crop performance was scored for a number of morphological traits, abiotic and biotic stresses. Resilience to biotic stresses is presented and discussed in correlation to yield and seed quality. The implications of these findings will be discussed to assess legume genetic diversity phenotyping for the improvement of crop, plant ideotypes.
We aimed to test if repeated early abiotic stresses could improve heat tolerance during anthesis heat stress in wheat cultivars. Two wheat cultivars, Gladius and Paragon, were subjected to a pre-anthesis high temperature priming process at three and five complete developed leaves stages. Primed and control plants were subjected to either a high temperature stress or non-heat stress temperature for 7 days during anthesis. Gas exchange and chlorophyll fluorescence were used to investigate the physiological performance of plants. No difference in assimilation rate was observed between treatments for Gladius. Heat stressed Paragon parameters were not measurable due to the premature senescence of plants. No strong evidence was observed to prove the initial assumption of early heat stress being accountable for improving heat tolerance. However, a great difference between cultivars in response to heat stress was observed. Yield parameters of Gladius primed plants did not differ from their respectively control treatment. A distinct result was observed for heat sensitive cultivar Paragon, suggesting a cumulative deleterious effects caused by the repeated heat stress.
Among different sensor technologies, hyperspectral imaging can assess the phenotype of plants non-invasively. For a deeper understanding of sensor data, the spectral information has to be connected with the plant phenotype and genotype.

Plant resistance reactions e.g. in incompatible barley-powdery mildew interactions, are based on a cascade of specific activities of genes, proteins, hormone signaling and in consequences on histological changes of the plant tissue. It is possible to determine and to distinguish different resistance reactions of barley genotypes against the powdery mildew pathogen using a hyperspectral-imaging microscope. Nevertheless, the relationship of molecular and biochemical mechanisms to the hyperspectral reflectance during compatible and incompatible barley-powdery mildew interactions are rather unclear.

The present study was conducted with Hordeum vulgare cv. Ingrid wild type (WT), a mlo based resistant (papilla formation) near-isogenic line, a Mla based resistant (hypersensitive reaction) near-isogenic line of cv. Pallas and the Blumeria graminis f.sp. hordei (Bgh) isolate K1. The hyperspectral reflectance was measured every 3 hours until 48 hours after inoculation (hai) followed by daily measurements until 120 hai to assess early interaction sites. In parallel, gene expression profiling of seven genes related to cell metabolism, signaling and resistance response was used to determine the powdery mildew pathogenesis and barley resistance reactions. For linking gene expression profiles with spectral profiles the Relief algorithm was applied. According to the function of tested genes during barley-Bgh interactions, relevant wavelengths were identified from 0 until 72 hai. These results enable for the first time a link between gene expression and spectral reflectance during barley-Bgh interactions, which can be used for efficient non-invasive characterization of the resistance type.
Cereal-intensive crop rotations like cereal and cereal species monocultures, and cereal rotations with one break crop within five years dominate high-latitude agricultural systems, while the share of fields with diverse crop rotations is <2%. Monoculture systems do not only reduce biodiversity, but lack resilience to weather variability and increase sustainability gap through e.g. abetting soil compaction and increasing dependency on crop protection. Diversity can be implemented into agricultural systems at different scales, but its impacts on resilience to weather variability and extreme weather events may vary accordingly. Incorporating genes that provide resistance against pests and diseases or improve NUE, WUE or general robustness makes novel cultivars better able to cope with stresses. When introducing such cultivars into cropping systems, one should not further reinforce their monoculture cropping or resistance and resilience are lost. When shifting from plant to crop stand scale, intercropping is the means for diversification (cultivar and species mixtures, population cultivars). When shifting to farm scale, farmer can improve resilience to weather constraints by having diverse crop rotations provided that spatial heterogeneity is primarily implemented instead of temporal heterogeneity. As an outcome of all these activities at different scales more diverse agricultural land use and landscape structures are achieved, which is essential for climate smart agriculture, but also biodiversity per se. Diversification activities that e.g. favor more diverse farmland fauna may provide various ecosystem services that further improve sustainability and resilience in agriculture. Hence, we underline that phenotyping diversity at all scales from genome to landscape is needed to demonstrate how diversification measures at one scale give support for other scales, and how they all impact resilience of agriculture to climatic constraints that are more frequent in the future.
Reproductive thermotolerance in tomato - pollen are key

Ivo Rieu.
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Heat wave-like temperature regimes negatively affect growth and fertility of plants and thus pose a severe risk to human food security. Given the expected further increase in global temperatures, a better understanding of these problems is urgently needed. I will show that production of viable pollen is a major limiting factor for fertility of tomato under long-term mild heat. I will present data on the molecular and physiological effects of high temperature and on the genetic determinants of pollen thermotolerance. Finally, implications for crop improvement are discussed.
European agriculture anticipates an unprecedented combination of stress factors, production threats and quality needs due to climate change. Various regions of Europe will be affected differently. Barley & wheat domestication, and landrace formation in Europe, were under very different climates than those emerging now. Alleles needed for sustainable, resilient, quality yields in a changed climate are likely not combined in current haplotypes of elite barley cultivars. These alleles are likely found in diverse landraces and wild relatives in the Mediterranean basin and Fertile Crescent -- areas that prefigure expected climate change. New precision, high-throughput phenotyping tools are essential to find trait-allele associations needed for future-climate breeding. In project ClimBar, under the framework of FACCE ERA-NET Plus JPI on Climate Smart Agriculture, we are interested in identifying the components of stress resilience, particularly for heat/drought stress. To do so, we are carrying out physiological phenotyping of lines from a barley core collection on three different scales control, and detail: field stomatal conductance; precision lysimeters in a glasshouse; gas exchange chambers. The goal is to understand drought response tradeoffs and mechanisms on the physiological and genic levels and to relate these to field phenotype and yield parameters.
21 - The importance of phenotyping legumes at multiple levels in order to mitigate climate change scenarios and help address sustainable development goals.

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As global population is growing, and the world economy continues expanding, humanity needs to think of creative ways to address societal challenges of food security, sustainable production and climate change. Legume grains may be a powerful tool to address these challenges in a collective way. However, they are underutilized and their role on environmental diets is still overlooked in Europe. Phenotyping at multiple levels, e.g. for nutritional quality, resistance to stresses, yield, or N fixation ability, is a critical step in order to leverage legume utilization as new ingredients or as participants in novel diversified cropping systems. Here we will highlight the importance of legume phenotyping to help solve global problems of climate change and nutritional security, while addressing sustainable development goals. We report on different germplasm collections of chickpea, faba bean, grass pea, lentil, lupin, pea and vetch which have been phenotyped according to their physical and quality parameters, an important step for novel food and feed development. Significant differences in 100-seed weight were found, ranging from 31-50 g in chickpea, 41-105 g in faba bean, 5-36 g in grass pea, 2-5 g in lentil, 29-47 g in lupin, 9-31 g in pea and 4-6 g in vetch. When looking at protein content, the coefficients of variation between genotypes were in general lower than 12% and, while lupin seeds showed the highest average protein content (average of 36.4 %), chickpea seeds had the lowest (average of 20.2%). The ionome of each genotype was also characterized, where the concentration of key nutrients like iron, zinc, phosphorus, magnesium, among others were determined to help in the identification of outstanding genotypes. Besides their nutritional quality, the phenotyping of legumes into novel cropping systems and short supply chains is under way in scope of the H2020 TRUE project, and a few success examples of this will be showcased.
Homogeneity investigation of red, purple and yellow maize using lipid profiling

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Homogeneity of the lipid profiles of the samples of red, purple and yellow maize was investigated using a GC-MS system with multivariate data analysis. Investigated samples of red, purple and yellow maize were obtained from the Maize Department of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Samples were ground into flour using a laboratory mill. Lipid components were extracted using n-hexane and fatty acids were derivatized into corresponding fatty acid methyl esters using a derivatization reagent TMSH (trimethylsulfonium hydroxide, 0.2 M in methanol). Derivatized extracts were then analyzed on a GC-MS device. The following mass fragments were chosen as representatives of eluting dominant fatty acids: 74 m/z for hexadecanoic acid, 67 m/z for 9,12-octadecadienoic acid (isomer), 55 m/z for 9-octadecenoic acid, 69 m/z for 9,12-octadecadienoic acid (isomer) and 74 m/z for octadecanoic acid. These mass fragments had the highest signals in mass spectra of corresponding fatty acids, which were the most abundant on obtained chromatograms. Surface areas of the ion peak functions were integrated and obtained values in a form of numerical matrix subjected to multivariate analysis. Hierarchical clustering was performed using a paired group algorithm and correlation similarity measure. The obtained dendrogram showed a high degree of similarity between the samples of red and yellow maize of about 0.8 and a clear distinction of the sample of purple maize with a degree of separation of about 0.2.
The potential and challenges of UAV-borne remote sensing for detection of potato late blight in the field

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In Sweden about one fourth of all fungicides used in agriculture is applied to potatoes (Solanum tuberosum), mostly to protect against late blight caused by Phytophthora infestans. Currently potato farmers are advised to manually scout their fields for late blight, a task that is time consuming. It would therefore be of great value to automate this process. Furthermore, automated detection could benefit field trials since it can be carried out more frequently, over larger areas, and be more objective, than if done manually. Photography, or multi-spectral reflectance measurements, using an unmanned aerial vehicle (UAV) is an attractive option.

The aim of the present project was to test the potential to detect, monitor and quantify infestation of late blight in potatoes by UAV-borne remote sensing. The tests were carried out in potato field trials in Southern Sweden over two seasons. One digital RGB camera and one multispectral camera with five narrow bands: blue, green, red, red edge and near IR (RedEdge, MicaSense). Images were collected from 8, 14 and 44 m height above ground at eight occasions during the growing seasons 2016 and 2017. Remote sensing data was extracted for the trial plots and paired with the infection levels observed by manual inspection. So far, we are able to associate a visible injury covering 4% of the canopy with a decrease in the red edge reflectance.

In a parallel attempt, we use computer vision and machine learning to detect late blight spots. To this end, we use the image recognition service Watson in collaboration with IBM. We are currently evaluating influences of confounding effects such as in field light variation, influence of soil and lesion size, and are creating a pipeline for automated handling of the images. This work is part of the EnBlightMe project (Vinnova 2016-04386), in which we develop an intelligent support system as a prototype app to help the farmer to detect late blight and avoid unnecessary spraying.
The recently introduced legislation on the registration of plant protection products (PPPs) has resulted in the significant reduction of the available PPPs. The latter, in combination with the ineffectiveness to develop PPPs exhibiting new mode(s)-of-action (MoA), has intensified the issues that the crop protection sector is facing. The situation becomes even more complex taking into account other important factors such as, unpredictable plant disease outbreaks, the development of PPPs-resistant populations of phytopathogens, the detection of PPPs residues in agricultural products, and toxicity issues of PPPs to non-target organisms. Within this context, the development of new types of formulation/active ingredients could provide solutions to the abovementioned issues. Nanotechnology is among the most important developments towards this direction, through its application in the formulation of PPPs and the use of nanoparticles as PPPs per se. Here, we have undertaken the task of evaluating the potential of innovative nanomaterials as next generation nano-PPPs (fungicides). In a first step, their phytotoxicity needs to be studied, and based on their complementariness, phenomics and metabolomics were selected for this purpose. The aquatic microphyte Lemna minor L. was used as the model biological system. In high-throughput bioassays, Lemna plants were exposed to various concentrations of different nanomaterials and their responses were recorded at the phenome level using a thermal imaging camera and at the metabolome level applying metabolomics. The effects of the applied nanomaterials on the metabolism of plants were combined with results of thermal imaging analyses providing an accurate evaluation of the plants’ stress levels. Results confirmed the applicability of the integrated high-throughput phenomics-metabolomics analyses, providing information that could be further exploited in PPPs? R&D for the selection of nanomaterials with the desired phytotoxicity.
Drought affects more people than any other natural disaster. It has been estimated that half of the world population will live in areas of high water scarcity by 2020 that will likely pose an increasingly dramatic problem of food shortage.

Haberlea rhodopensis is a paleolithic tertiary relict species best known as resurrection plant with remarkable tolerance to desiccation. H. rhodopensis exposed to severe drought stress shows the ability to maintain the structural integrity of the photosynthetic apparatus which re-activates easily upon rehydration. In addition to its homiochlorophyllous nature, H. rhodopensis with such capability of resurrection is a trait of significant importance in the global climate change.

The NGS sequencing and annotation analysis of H. rhodopensis cp genome uncover several intriguing features which can be used as a base to understand the resurrection tolerance of this plant. Specifically, the H. rhodopensis cp genome harbors 137 genes, of which 86 protein-coding. The site-specific selection analysis points out positively selected sites in several chloroplast genes such as atpE, rbcL, psbl, psbA, ndhH and accD. The observed specific cp genomic features of Haberlea rhodopensis may be interpreted as being a consequence of molecular adaptation to drought stress, which awards an evolutionary advantage to this species.

The chloroplast genome reported in this study will allow understanding the function of the specific sites under selection by developing site-directed mutagenesis assays or by developing point mutations at those sites, which can be a step and bridge to bioengineering of drought-tolerant crops.

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26 - Field phenotyping of drought stress effects in wheat genetic resources using chlorophyll fluorescence and spectral reflectance records.

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One of the challenges of phenotyping is to characterize the drought responses of numerous genebank accessions in the field. To examine this issue, we tested the set of 25 winter wheat genebank accessions grown in field trials in two variants: the irrigated variant and the variant without irrigation. Because of very low precipitation during the vegetation period, the plants grown in non-irrigated plots were exposed to the significant water deficit. During the spring season, the plants were monitored weekly using fast non-invasive methods based on proximal sensing or fast leaf-clip measurements, such as VNIR polyspectral and hyperspectral measurements, chlorophyll fluorescence (analysis of fast chlorophyll kinetics and fluorescence excitation ratio method). The leaf samples were collected for analyses in the laboratory (SPAD analysis, hyperspectral imaging, RGB scanning, photosynthetic pigment analysis).

Our results demonstrated that estimations of chlorophyll contents based on spectral reflectance and fluorescence excitation ratio were more reliable when done in laboratory conditions on detached leaves compared to field recordings. On the other hand, some of the numerous parameters derived from field measurements were able to recognize drought effects, even in the early stages of drought stress, with significant variance across the testing germplasm. The statistical analysis of the effect of the factor "irrigation" identified that especially the ratio of far-red to red fluorescence (F730/F685, i.e. SFR parameter of excitation ratio method) based on proximal sensing in the field and parameter normalized area (Sm) derived from fast fluorescence field records using leaf clips seem to be very promising for assessment of the drought stress effects on photosynthetic apparatus in wheat genetic resources. The study was supported by the national grants APVV-15-0721, APVV-15-0562, VEGA-1/0923/16, VEGA-1/0831/17, and by the UE H2020 project No 731013 (EPPN2020).
27 - Monitoring of cereal crop growth dynamics by stereoscopic vision and machine learning techniques.

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In the context of plant breeding and precision agriculture, the characterization of phenotypes and their evolution still constitutes a bottleneck. Joining the global effort to bridge the genotype-to-phenotype gap and to take on the challenge of feeding the ever growing world population, the aim of this study is to focus on the measurement of plant architecture dynamics in cereal crops at the plant scale.

A stereoscopic vision system was designed to reconstruct 3D scenes from high-resolution color images. This equipment was tested in field conditions and will be used in fully controlled chambers of an ecotron facility (TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech) to characterize the vegetation response to simulated future climates.

Data analysis methodology is oriented towards state of the art machine learning techniques in order to extract the evolution of morphological traits at the scale of the plant. Expected challenges in the data treatment workflow are data management, pre-processing and organ segmentation. A prototype of the phenotyping pipeline is proposed.
28 - Phenotypic correlations between image-based morpho-physiological traits and dry biomass at vegetative stage in maize.

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Twenty maize inbred lines were studied for their response to water stress, nitrogen deficiency, and their combination during vegetative stage. The plants were grown in a greenhouse under optimal conditions until V6 growth stage. From V6 stage plants were subjected to stresses and imaged for four weeks at the automated phenotyping and imaging platform at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). The experiment consisted of four treatments: 1) control - optimal watering and fertilizing; 2) nitrogen limited (N) - optimal watering; 3) water limited (W) - optimal fertilizing; and 4) nitrogen and water limited (N+W). At the end of the experiment dry biomass weight (DW) was manually measured and correlated with digital traits. Out of nearly 300 parameters obtained through an image analysis pipeline, 25 informative phenotypic traits were extracted and classified into three categories: biomass-related (3), architectural (12) and physiological (10). Estimated digital volume was highly correlated (0.77) with DW in optimal conditions, while somewhat lower, but still significantly correlated in N (0.66), W (0.58) and W+N (0.55). Architectural traits mostly showed different correlation patterns with DW according to the treatment, with correlations decreasing in W and W+N. Plant height varied the most, being significantly positively correlated with DW in optimal and N limited conditions (0.60 and 0.54, respectively) while negatively correlated in W and W+N limited conditions (-0.26 and -0.18, respectively). Solidity (used as a proxy for leaf area index) was the only architectural trait negatively correlating with DW in control and N treatments, but positively correlating in W and W+N. Physiological traits showed weak or no correlations at all with DW in all treatments. Principal component analysis has also been done to further explore the relationship of chosen traits and their relevance for genotype discrimination in different environments.
Nitrogen management for open-field production of ornamental plants is challenging due to the large number of species and cultivars. Sub-optimal nitrogen nutrition negatively influences the commercial quality of the plants while supra-optimal N has an environmental impact due to nitrate leaching. Therefore, (a) reliable indicator(s) of plant nitrogen phenotyping is/are needed.

Sensors based on the optical properties of chlorophyll have already proven their use for plant phenotyping in various agronomic cropping systems. They can be used as quick, nondestructive and relatively inexpensive tools to support decisions concerning in-season N fertilization. Research on these optical N sensors in outdoor ornamentals is scarce. For this study, 4 woody perennials were chosen, Acer pseudoplatanus, Ligustrum ovalifolium, Prunus laurocerasus ‘Rotundifolia’ and Tilia cordata, as well as 2 herbaceous species Chrysanthemum morifolium and Begonia x tuberhybrida. All plants received 3 different N treatments in 3 replicates. The experiment was first conducted in 2016 and repeated in 2017.

The leaf and whole-plant nitrogen content and non-destructive measurements were monitored regularly during the growing season. At leaf level, the SPAD-502 chlorophyll meter was evaluated. At crop level, the species were monitored with a GreenSeeker RT100 (Trimble) which calculates the normalized difference vegetation index (NDVI). This canopy reflectance index can be correlated with N and biomass. Early results show significant correlations between foliar nitrogen and SPAD values for Begonia, Ligustrum, Acer and Tilia, while for Prunus and Chrysanthemum correlations were absent. At canopy level, only for Acer, Ligustrum and Chrysanthemum a correlation was found between NDVI and N in 2016. In 2017 this relation was less clear. Although NDVI was an estimator for biomass for Acer, Chrysanthemum, Prunus and Tilia, robustness is further investigated.
In crop genetic studies, the mapping of longitudinal data describing the spatio-temporal nature of agronomic traits can potentially elucidate the factors influencing their formation and development. Here, we combine the mapping power and precision of a MAGIC wheat population with robust computational methods to track the spatio-temporal dynamics of traits associated with wheat performance. NIAB MAGIC lines were phenotyped throughout their lifecycle under smart house conditions. Growth models were fitted to the data describing growth trajectories of plant area, height, water use and senescence and fitted parameters were mapped as quantitative traits. Single time points were also mapped to determine when and how markers became and ceased to be significant. Assessment of temporal dynamics allowed the identification of marker-trait associations and tracking of trait development against the genetic contribution of key markers. We establish a data-driven approach for understanding complex agronomic traits and accelerate research in plant breeding.
31 - Integrating phenotyping and modelling in an oat collection for speeding resilience under current and future Mediterranean climates.
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Oat (Avena sativa L.) is an important cereal crop. During the last 20 years, the oat cultivated area in the Mediterranean rim has steadily increased by approximately 7500 ha per year. This almost equalled the oat cultivated area between Northern Europe and Mediterranean rim. However, the oat yield in Northern Europe remains much greater than that of the Mediterranean area (approximately 2.7-fold), because it is not particularly well adapted to hot and dry weather. As a consequence, there is a need for breeding oats for resilience to current Mediterranean and future climate conditions. Two of the bottlenecks in breeding are the availability of diverse genetic resources and appropriate selection tools. To speed breeding of resilient oats under Mediterranean conditions, we performed a field phenotyping of an oat collection consisting on 707 cultivars and landraces, under Mediterranean environments. Accessions were mapped in a virtual map, recording site and year of harvest, morphological and agronomical traits. Phenotyping was carried out in 3 different environments at two contrasting locations in Spain. To evaluate the influence of environmental factors on the agronomic traits, 9 climate variables (maximum temperature, minimum temperature and rain during pre-flowering, flowering and post-flowering period) obtain from the AEMET database for each location were combined with agronomical data and subjected to non-metric multi-dimensional scaling ordination. This ordination technique reduce the climate variable matrix prior modeling each agronomic trait. Canonical correspondence analysis (CCA) was then performed to determine the relative impact of the selected climatic variables on agronomic traits and genotype performances. The knowledge of the impact of the climatic variables in the different accessions will help to improve the oat crop for adaptation to current and future Mediterranean growing conditions.
32 Grapevine phenotyping in resistance/susceptibility to Plasmopara viticola
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The cultivated grapevine Vitis vinifera is prone to several diseases, being downy mildew disease, caused by Plasmopara viticola, one of the greatest threats to modern viticulture. As a control measure, several fungicide applications are necessary every year. This practice has several associated problems, from the environmental impact to the economical level, and even in human health. One of the most urgent needs in today’s agriculture is the reduction of phytochemicals used to control pests and diseases. In order to achieve it, the identification of resistance/susceptibility-related biomarkers is crucial.

We conducted two approaches in order to phenotype grapevine resistance. The fatty acid content and pigment profile of different grapevine species and cultivars was analysed and a clear separation between resistant and susceptible accessions was obtained. Total fatty acid content was significantly higher in susceptible genotypes, and the total amount of pigments followed the same trend. A higher linolenic (C18:3) to linoleic (C18:2) acids ratio was found in the resistant genotypes, together with lower chlorophyll a to b ratio. In addition, the Double Bond Index (DBI) was found to be higher in the susceptible cultivars. Leaf metabolome of several resistant and susceptible grapevine accessions was analysed by ESI-FTICR-MS. Around 800 metabolites were characterized in the different resistant and susceptible genotypes leading to the identification of some putative biomarkers.

Taken together, the collected data indicates very promising way to an early screening for resistance/susceptibility traits in grapevine breeding programs.
Woody plants are usually perennial plants in which the activity of the lateral meristems leads to extensive production of secondary growth tissues (wood/secondary xylem and cork/phellem) in stems and roots. As a result, a set of characteristic traits related to secondary tissues can be described in these plants. The gap in current plant ontologies for describing such traits led us to propose a new ontology, the Woody Plant Ontology (WPO), for describing all variables used during woody plant observations. This ontology covers several domains of knowledge in woody plant biology, from the agronomic down to the biochemical level, with the goal of standardizing and organizing trait information. This will facilitate the integration, management and analysis of woody plant data, thus promoting its FAIRness.

The WPO is integrated in the Crop Ontology (CO) framework, which includes species specific ontologies describing traits, methods and scales (http://www.cropontology.org/). Building on past and ongoing projects at national and international levels, a list of all variables and terms of woody plant observed traits used by the researchers and breeders was integrated during the development of the WPO. WPO currently describes 197 traits organized in 12 classes. As an example, Morphological traits include 'Bark Roughness', 'Tree Ring Width', 'Wood Density' while Biochemical traits include 'Cellulose Wood Composition', 'Syringaldehyde/Coniferaldehyde Wood Contents'. All the traits were curated taking into account several aspects such as synonyms, the context of use, the scale, among others.

The future work will be the incremental validation and refinement of the ontology, which involves adding methods of trait measurement and experiments, and integrating information from various sources envisioning the use of these information in a Breeding Platform.

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Increase of photosynthetic capacity of major crops can contribute to the improvement in biomass production and crop yield. Phenotyping of genetic resources concentrated in genebanks is expected to provide germplasm useful for breeding programs. To examine links between photosynthetic performance, leaf traits and results of fast optical parameters, we have realized measurements in a collection of 25 diverse wheat genotypes selected from hundreds of accessions of Slovak Genebank at NAFC-RIPP Piestany, Slovakia, to ensure diversity of leaf traits. Plants were grown in pots outdoors and in post-anthesis growth stage, the simultaneous gas exchange and chlorophyll fluorescence (A/Ci curves, light response curves) and Dual-PAM fluorescence / PSI transmittance were measured to monitor photosynthetic capacity and photoprotective responses of the genotypes. In addition, in each measured leaf, the fast, non-invasive methods were applied using HandyPEA and Multiplex-3 fluorescence sensors, chlorophyllimeter SPAD. After measurement, the leaf was detached and analyses of chlorophyll and carotenoid content and dry mass per leaf area (LMA) were performed. In parallel with pot experiments, the field trials with the identical set of genotypes were performed, in which also field measurements of hyperspectral reflectance were done in parallel with non-invasive and destructive measurements of leaf traits. The results of gas exchange analyses have shown that genebank accessions differ in photosynthetic capacity and photoprotective responses. A higher photosynthetic rate was not associated with chlorophyll content nor with a leaf thickness (LMA). On the other hand, some of the parameters calculated from the fast fluorescence records seem promising in estimating photosynthetic performance, but further examination is needed to confirm reliability of the methods. The study was supported by the national grants APVV-15-0721, VEGA-1/0831/17, and by the UE H2020 project No 731013 (EPPN2020).
Heat stress is one of the main abiotic constraints to plant biomass production. To foster adaptation to climate change it is essential to understand how crop plants respond to a warmer climate. The collection of large-scale plant phenotypic data to characterise specific traits associated with biomass production can be useful for the selection of more productive genotypes. Furthermore, the association between phenotyping data and putative biological functions can lead to a better understanding of the underlying mechanisms that control tolerance to increased temperature.

Thus, using phenotyping techniques, we quantified the variation in traits associated with biomass production across cultivars of wheat and maize, under drought and increased temperature. Photosynthesis and growth rate will be used as phenotypic indicators for adaptation to a warmer and drier climate of different varieties of maize and wheat with putative resistance to heat stress.

Plants were grown under controlled conditions and non-invasive techniques were used to comparatively analyse the effect of increased temperature. RGB, NIR and thermal images were collected in different developmental plant stages and the in vivo photochemical performance of PSII was assessed by rapid fluorescence induction curves (JIP-test) and Imaging Pulse-Amplitude-Modulated fluorescence (Imaging-PAM). Photosynthetic gas exchange was measured simultaneously with Imaging-PAM fluorometry.

Machine learning techniques will be used to analyse the data obtained with the JIP-test and multivariate statistical methods will be used for the exploration and integration of the different biological data sets.
36 - Multivariate tools for plant phenotyping

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One of the major bottleneck of PP consists in the way information about genotype and phenotype are merged in order to extract knowledges to be used for plant research, breeding, product development, and quality assessments. At CREA-IT we developed and applied multivariate statistical tools also for PP. This short note aims to provide a quite comprehensive panorama of the multivariate statistical approaches to be used for this purpose. Genetic information can be obtained by various molecular markers, such as DNA seq. and SNPs, or from the whole genome thanks to NGS. Phenotype variables could derive from agronomic variables, disease resistance, shape, color or texture of roots, fruits or entire plant, volatilomics variables, etc.. Multivariate statistical approaches could be classified as unsupervised or supervised methods. Multivariate ordination techniques are unsupervised techniques, among ordination techniques the ones which take into account two kind of different matrices are CANOCO, 2-BPLS or MDS. Supervised techniques could be divided into regression, classification and modelling techniques; those approaches are useful to predict an a priori known variable. Regression techniques aim to predict a quantitative variable. Classification analyses answer to the general question: ?Which is the most probable category in which a given object O could belong?? This general question expects the presence of at least two categories and the object could belong to one and just one group. Modelling techniques answer to the general question of whether an object O, stated of class A, really belong to class A. Modelling approaches calculate the ?prediction probability? with a classification threshold for each modelled class. Using a class-modelling approach, it is possible to attribute objects not only into one or more classes, but also to none (i.e., outlier). At least, ANNs are particularly useful in performing for nonlinear responses (for regression or classification purposes).
Climate change imposes increasing restrictions and risks to Mediterranean viticulture. Heat and drought stress are becoming more frequent and extreme and put in risk sustainability of Mediterranean viticulture. In addition, row crops such as vineyards, are more vulnerable to heat stress due to the amplified effect of soil surface on vineyard’s heat fluxes which can be harmful for leaf and berry physiology. Leaf/canopy temperature is a biophysical variable with high physiological and agronomic meaning. Therefore, an improved understanding on the spatial and temporal dynamics of leaf/canopy temperature (thermal microclimate) in individual plants and the vineyard can support more precise vine’s phenotyping and management. In this work we propose a conceptual approach to describe and integrate the major interactions occurring between vines, soil and atmosphere focusing on the impact of soil/plant water status and air temperature on vine’s temperature, gas exchange, phenology, and berry yield/composition. Ultimately a conceptual model will be build up to support decision making in irrigation to control heat and water stress. This model will help to develop decision support systems for crop and soil management in irrigated Mediterranean vineyards.

Key words: Mediterranean viticulture, temperature, DSS, water and heat stress, soil and canopy temperature, irrigation
38 - Analysis of leaf morphological traits of the European hornbeam (Carpinus betulus L.) in Banja Luka region (Bosnia and Herzegovina).
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Many software tools were developed for analysis of leaf morphological traits, of which most are freeware, and can be successfully applied in agricultural research. European hornbeam (Carpinus betulus L.) is present in woods of the Banja Luka region, mostly in associations with Quercus robur L. as part of the primordial vegetation. Unfortunately, its production in local horticultural nurseries is almost non-existent, eventhough this species has great potential for use in urban environments, and so the quest for the source of high quality reproductive material from local populations is one of the aims of this research.
Leaves were taken from preselected mother trees in July 2017 from 3 localities (Krupa na Vrbasu, Kadinjani and Donji Podgradci). A total of 420 leaves were pressed, scanned (HP ScanJet 4030p, 600 dpi), their images calibrated and measured by image analysis tool MorphoLeaf v. 1.41. Analysis in MorphoLeaf is based on semi-automatic identification of leaf geometric landmarks (such as leaf base, tip, serration points). For biometrical and statistical comparison of data standard descriptive measures were used in combination with factorial anova and appropriate post-hoc testing (p
Drought events and low phosphorus (P) availability are major limitations for rainfed rice production in sub-Saharan Africa, and they often co-occur. In search for sustainable and resilient solutions to increase rice production, it is important to unravel the ‘belowground opportunities’ of the rice crop. Root architecture and root anatomy are expected to respond to both water and P availability. A main concern in this regard is that genotypic variation in response to one of both factors (water and P) can be less or more important than the responses to another factor whereby the perceived beneficial root traits of a genotype ‘tolerant’ to one of both factors can diminish or even disappear when the other factor varies.

For this study, two pot and two field trials were conducted in Tanzania in order to evaluate anatomical and architectural root responses of contrasting rice varieties to combinations of different levels of P (deficient to non-limiting) and water availability (water stressed to submergence). Root development was analyzed by both manual and semi-automatic methods and it was then linked to the drought and/or low P tolerance of rice varieties.

The observed genotypic variation in root traits provides opportunities for trait-based breeding. The plasticity of several traits to multiple environmental factors highlights the need for strategic trait selection and considering interactions of multiple stress factors seems crucial before exploiting root traits in trait-based breeding programs. Therefore, there is a strong need for enhanced root phenotyping technologies that can be used in field conditions. Further root studies on enhanced nutrient uptake in rice should take into account the strong modifying effects of water availability.
Elevated atmospheric CO2 (eCO2) is known to affect plant growth and nutrition leading to reduced mineral or protein concentrations in several crop species, and these losses will worsen in the near coming future. Soil iron (Fe) deficiency in calcareous soils, which represent about 30% of the arable land in the world, highly affects plant growth leading to reduced yields and nutritional quality. Legume crops provide a large share of the global population's diet and will be affected by eCO2 and Fe deficiency, but there are no studies linking the effect of these two stresses. Aiming at understanding the interaction of these effects in two legume species - soybean (Glycine max) and common bean (Phaseolus vulgaris) - we conducted a short-term study where plants grew under ambient CO2 (400 ppm) and eCO2 (800 ppm) in hydroponics using nutrient solution with (20 uM) and without Fe. At the end of the experiment chlorophyll levels, photosynthetic rates, ferric chelate reductase (FCR) activity, dry weight of roots, shoots and leaves, mineral composition and organic acids concentrations of roots and leaves were measured. Plants of both species grown under Fe deficiency showed symptoms of Fe deficiency chlorosis, with 40-65% lower chlorophyll levels. Also, in both species, FCR activity was generally higher in plants grown under eCO2 but for soybean the difference was significant in the presence of Fe (four-fold higher) whereas for bean it was significant in the absence of Fe (ten-fold higher). Leaf and root biomass were also affected by these stresses with eCO2 combined with Fe deficiency resulting in significantly lower leaf and root biomass in the plants of both species. Nutrient composition and organic acid analysis allowed us to further understand the underlying impact of these combined stresses. Our results clearly show an interaction of both factors leading to different plant behaviors which may be plant species dependent.
In Mediterranean climates, vine’s water status and air temperature are major drivers of growth and berry ripening. Management of mild water stress in grapevine demands a continuous and precise regulation of the water supplied as function of vine’s phenology. Thus, assessment, in real time, of major environmental (climate, soil water content) and plant indicators (vegetation appearance and physiological variables) are on demand. Vineyard water management should be focused on precise grapevine water stress management in order to avoid excessive vigor, optimize fruitfulness, yield and berry size and composition and save water.

Plant water transport continuum pathway (soil-plant-atmosphere) is subjected to several resistances (soil-root interface, xylem hydraulic, stomatal and aerodynamical resistances) that couple leaf transpiration to soil water content and atmospheric water demand. Under conditions of reduced transpiration, as usually occurs under darkness and low air vapor pressure deficit (VPD), stomata tend to be closed and plant’s water potential reaches an equilibrium with soil water status at root’s active water uptake layer. In these conditions, the predawn leaf water potential (ΨPD), works as a robust proxy of soil water potential. However, ΨPD assessment is a destructive, time consuming and intensive labor methodology, with major constraints in terms of spatial and time resolution. In this context, a robust and easy to assess, grapevine water stress indicator is strongly requested for vineyard deficit irrigation management. In this study, we test the possibility of using soil water content, air temperature and VPD, as grapevine water status estimators.

Keywords: grapevine phenotyping, grapevine water relations, predawn leaf water potential estimators, soil water content, VPD
Optimization of algal metabolism toward the improvement of bioproduct production remains a challenge that requires experimental strategies at the level of cellular phenotyping. The objective of our study was to evaluate photosynthesis, fatty acids composition and lipid metabolism genes in two strains of the microalga C. reinhardtii to improve lipid production. Comparative analyses of the two strains, one mutated (displaying a low CO2 assimilation capacity) and a non-mutated control, were undertaken in mixotrophic conditions. Differences between the two strains were assessed with phenotyping tools that comprise nondestructive methods such as PAM (Pulse Amplitude Modulated) Fluorometry and Clark-type electrode and biochemical methods such as gas chromatography and quantitative PCR.

Our results showed that the mutated cells had divergences in photosynthetic phenotype with a significant decrease in O2 evolution and in the efficiency of photosystem II. Using a phenomenological energy fluxes analyses of the fluorometric parameters, mutated cells exhibited a low number of reaction centers (RC/CS), significant decreases in the amount of energy absorbed (ABS/CS), entrapped energy (TRo/CS) and electron transport flux per cross section (ETO/CS) but a higher energy dissipation (DIo/CS). On the other hand, the respiratory O2 consumption was significantly higher in mutated cells compared with the control. The fatty acid composition and contents also varied between the two strains. The expression of genes related with lipids accumulation such as diacylglycerol acyl transferase (DGAT) was up regulated in mutated cells indicating induction of storage lipids. The desaturases CrDES, with ω3 desaturase activity, and the plastid Cr FAD6 were down regulated which tie with the changes of fatty acids profile of the mutant. All data together of this cellular phenotypic characterization indicated that the mutant studied is a good candidate for storage lipids production.
Plant abiotic and biotic stresses impact yield and fruit quality. Hence, a premature identification of the stress helps to avoid quality and crop losses. Microorganisms such as bacteria and fungi often cause severe diseases, which in turn could be controlled by deploying pesticides. Botrytis cinerea is one of the most damaging necrotrophic fungi attacking hundreds of different plant species. In tomato, Solanum lycopersicum, it attacks leaves, stems and fruits. A presymptomatic identification could allow an efficient fungicide regime by applications only when the disease is present.

In artificial inoculation assays under controlled environmental conditions, we infected detached tomato compound leaves with B. cinerea and ran time-lapse experiments using a multispectral imager (RedEdge, Micasense, Seattle, WA, USA). This imager collects data in five 10-40 nm wide bands (red, blue, green, RedEdge and NIR (near infrared)) through individual lenses on one chip. After applying dark frame subtraction, lens de-vignetting, lens undistortion and intensity-based band alignment, an alignment error of two pixels remains due to parallax effects. A supervised RedEdge-based classification approach allowed for the presymptomatic identification of infected leaf tissue within 24 hours post inoculation (hpi). In fact, after manually tweaking classification thresholds we were able to identify diseased spots even earlier at approximately 16 hpi. Shapes of infected but presymptomatic tissue obtained with multispectral, low-resolution camera were then compared to RGB imagery of visible symptoms taken three days post inoculation. The results suggest a detection of roughly 75% of symptomatic leaf area by multispectral imaging in a presymptomatic phase (24 hpi). This approach is adapted to controlled environmental conditions. Future work will look at the challenges under day light conditions.
Plant phenotype is determined by the genetic background and environmental conditions. Interaction of the genotype and environmental factors influences plant growth and development, physiological and molecular traits. Characterization of phenotypes is crucial to understand the regulation of stress in responses to climate changes. Non-destructive analysis of plants through color imaging is an increasingly popular method to define growth parameters, characterize plant development in time.

We have developed a non-invasive method, which simultaneously measures basic morphological and physiological parameters of in vitro cultured plants such as Arabidopsis thaliana. Changes of plant size, shape and color is monitored by repeated photography with a commercial digital camera. Images are analyzed with the Matlab-based computer application PlantSize, which simultaneously calculates several parameters including projected rosette area (pixel area, fresh weight, convex area and ratio), and color (chlorophyll and anthocyanin contents). Numerical data are exported in MS Excel format. Subsequent data processing provides information on growth rates, chlorophyll and anthocyanin contents. Utility of the system is demonstrated by revealing small but significant differences between wild type and transgenic Arabidopsis plants overexpressing the HSFA4A transcription factor or the hsfa4a knockout mutant, subjected to different stress conditions. Morphological differences were revealed by comparing rosette size, shape and color of wild type plants with phytochrome B (phyB-9) mutant.

The developed technology offers a simple, affordable and fast way to measure several morphological and physiological parameters of Arabidopsis plants. The methods are based on non-destructive imaging allowing repeated measurements and monitoring changes of various growth parameters in time. PlantSize is publicly available (http://www.brc.hu/pub/psize).
During past decades, plant breeding has been performed under conditions of high fertilization and intensive crop protection, leading to a decrease of diversity among modern cultivars. The selection of genotypes under these conditions has also affected the ability of modern varieties to establish beneficial associations with soil functional microbial assemblages, like mycorrhizae fungi and symbiotic nitrogen fixing bacteria. The community structure of these microbial guilds is crucial for agricultural systems productivity, since they are facilitating nutrient uptake and crop tolerance to abiotic stresses, like drought and salinity, while in some cases they are providing protection from soil borne pathogens.

In our plant breeding program, it has been demonstrated that different genotypes of maize, cowpea and chickpea exploit differently the native microbial communities and this has been associated with important traits, like yield and N uptake. Crucial in these findings has been the application of a novel plant breeding paradigm, known as Prognostic Breeding that uses the individual plant grown at ultra-wide distances as the unit of phenotyping and selection, exploiting the unique properties of the honeycomb selection designs and a novel phenotyping equation.

In addition, it was found that the symbiotic nitrogen-fixing bacterial community identified in local cowpea and chickpea, was genotype-dependend, while significant interactions between nitrogen-fixing bacteria and mycorrhizal inoculum exist. Our results showed that genotypes with the most interesting plant traits were significantly correlated with associated functional microbial assemblages, like mycorrhizal fungi and nitrogen fixing bacteria.

The ultimate goal is to better understand how the selection process influences the plant-associated microbial functional communities and how these interactions can be used to select for improved plant productivity traits in changing environments.
46 - Associating easy-to-quantify stress markers at cell and leaf level under progressive drought in the lab.

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Several physiological parameters can be quantified to date in order to evaluate water stress levels in plants. Some of them are more time-consuming and/or labor-demanding than others. In addition, some of them are destructively-obtained, while others can be obtained without damaging/touching the plant. Modern studies are usually using different physiological parameters to quantify water stress levels at cell and/or leaf level. The diversity in the parameters used among studies makes the comparison between studies and/or plant species challenging. In this study, different physiological parameters such as leaf net photosynthesis, stomatal conductance, chlorophyll fluorescence, leaf relative water content, chlorophyll/anthocyanins/carotenoid contents, electrolyte leakage and lipid peroxidation levels were quantified during progressive drought in important plant species such as maize and wheat. The aim was to relate the different parameters and compare their relations observed in different plant species. Our results indicate commonalities as well as differences on the relations between different physiological parameters in different plant species and thus discriminate between parameters that can be securely used or not such as universal stress markers in order to describe drought stress levels across species and studies.
Uptake and bioaccumulation of three widely prescribed pharmaceutically active compounds in tomato fruits and mediated effects on fruit quality attributes.

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Pharmaceutically active compounds (PhACs) released in agroecosystems have been found to be taken up by and accumulated in the edible parts of crop plants. By employing simulated hydroponic cultivation under control conditions, the present study aimed at evaluating 1) the uptake and bioaccumulation of three common PhACs (diclofenac, DCF; sulfamethoxazole, SMX; trimethoprim, TMP), either applied individually or as mixture, in tomato fruits harvested from the first three fruit sets, and 2) the PhACs-mediated effects on fruit quality attributes. DCF was not detected in tomato fruits, whereas both SMX and TMP were detected in varying concentrations in fruits, depending on the time of harvest, the mode of application and the allocation of plants in the greenhouse. The studied PhACs applied at environmentally relevant concentrations did not significantly affect plant productivity. Nevertheless, important fruit quality attributes, such as SSC and carbohydrate (fructose, glucose, sucrose, total sugars) contents were significantly impacted, mostly by DCF applied individually, suggesting that DCF transformation products (TP) not quantified in tomato fruits may exert significant effects on fruit quality attributes. In addition, no additive or synergistic effects of the mixture of PhACs on studied fruit quality attributes were revealed. Gene expression analysis showed that PhACs-mediated effects on the carbohydrate content of fruits can be attributed, at least to some extent, to the significant modulation of the abundance of transcripts related to the biosynthesis and catabolism of sucrose, such as SlSuSys, SlLin5, SlLin6 and SlLin7. To our knowledge, this is the first report highlighting the potential effects of PhACs released in agroecosystems on the quality of widely consumed agricultural products. In any case, further studies are needed for the overall assessment of the potential impact of PhACs on the quality of agricultural products under real agricultural practices.
48 - Multi-omic datasets integration for flax abiotic stress research

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As part of the NoStressWall project aiming to better understand how flax plants, with high economical interest (textiles, bio-composites, isolation), are able to cope with water deficit, transcriptomics (3704 genes), proteomics (396 proteins), metabolomics (40 metabolites) and morphological (8 variables) data sets were generated for leaves, stems and roots, during a 10-day time-course study. By multi-block analyses with the block.spls function of the R package mixOmics (Le Cao et al., 2009; Le Cao et al., 2011; Gonzalez et al., 2012) we studied the relationships between the three different -omics data sets and the physiological one, in order to identify in each block which subsets of ‘active’ variables are in strong relationships with the other blocks. This method has the advantage of providing a wide range of linear multivariate methods for data exploration, integration, dimension reduction of large biological data sets. It also allows visualisation outputs (Rohart et al., 2017) which unravel complex associations between different biological entities and allow to better visualise the correlation structure between the different biological entities considered. This was performed in three different organs, roots, stems and leaves, therefore allowing the identification of variables being controlled by time-lagged values of other variables. By the way, we achieved a more precise understanding of the adaptation mechanisms of flax to osmotic/drought stress.
With over 2.25 billion cups consumed per day, coffee is one of the most important beverage crops in the world. It is cultivated across three continents (Africa, Asia, and America) where more than 80 million people depend on its income. The Arabica coffee has the best quality/aroma, yet it is highly susceptible to several pathogens; the most important is coffee leaf rust (CLR) caused by Hemileia vastatrix, a fungus widespread in all coffee growing countries (CGCs). The adaptive capacity of H. Vastatrix is leading to a gradual loss of resistance in some commercial cultivars in many CGCs. If no chemical control measures are taken, huge depreciation of marketing values are deemed to occur. However, increasing societal expectations for a more sustainable coffee production urges the replacement of pesticide treatments by alternative strategies. Plant resistance inducers (that elicit the plant’s constitutive defenses) have been developed at Universidade Federal de Lavras (UFLA) and at Instituto Nacional de Ciência e Tecnologia do Café in Brazil. The most promising results in CLR control were obtained with Greenforce CuCa (a formulation based on by products of the coffee industry). Furthermore, field applications of Greenforce CuCa revealed lower coffee defoliation and high productivity, not differing from fungicide treatments, but with lower ecological impact. Physiological (leaf gas-exchange) and biochemical (H2O2 metabolism) analyses of the GreenForce CuCa effect on coffee revealed changes at photosynthetic and respiratory metabolisms. Results showed that primary metabolism and redox homeostasis can discriminate between all treatments (GreenForce CuCa, Bion®, H. vastatrix). Although the infection by H. vastatrix caused a decreased in leaf conductance and carbon assimilation in all samples, the Greenforce CuCa impact was lower. We predict that plant resistant inducers will play a role in coffee production management.
Plants are sessile organisms with wide array of response pathways to meet the challenges caused by environment. Characterization of these response pathways holds a key to genetic resources that can be utilized in molecular breeding for agriculture that is facing new stress factors due to changing climate. Molecular plant responses manifest in pleiotropic developmental and physiological changes that affect plant growth, development and physiology. To study the interactions between phenotypic traits and defined perturbations we have developed several in vitro assays utilizing the National Plant Phenotyping Infrastructure (NaPPI) at the University of Helsinki site, Finland. These assays include applications of i) hormone gradients for testing genetic interactions in hormone signaling, ii) bioassays for testing allelopathic compound interactions with biological processes, and iii) plant pathogen infections on dissected organs. For example, mutant lines representing ABA response genes from ubiquitin proteasome system signaling pathway were phenotypically characterized on ABA concentration gradient. Seed germination as indicated by green cotyledon emergence was recorded daily with Chlorophyll fluorescence camera. Our results indicated three novel ABA response regulators that were up-regulated upon ABA treatment and were resistant to ABA during germination. We have also shown that the results obtained with NaPPI were as precise as those observed under stereomicroscope, indicating that NaPPI was capable and reliable for detecting Arabidopsis seed germination. Automation of seedling analysis greatly facilitated screening through several gradients of hormones and compounds. Furthermore, pathogen infection assays on dissected organs showed that fluorescence and thermal cameras detected disease symptoms earlier than observed by eye, while RGB camera was used to quantify chlorotic and necrotic tissues, all together facilitating assessment of dynamic symptom development.
Proteomics has played an important role in advancing our understanding of abiotic stress response mechanisms in a wide range of crops by giving important insights into dynamic changes in protein abundance and translational and post-translational regulations. The C-termini of proteins undergo general and distinct chemical modifications that serve as recognition signatures in a variety of cellular processes, such as protein sorting, integration of proteins into membranes, regulation of protein activity, and formation of macromolecular complexes. These molecular mechanisms are partly genetically controlled but are also strongly influenced by environmental factors such as water availability and temperature, etc.

In some fields there is renewed interest in analysing the C-termini of proteins in a proteome but this subfield remains largely unexplored in agronomy and crop science. Annotation of the carboxyl-terminal status of a proteome may facilitate functional classification, identification of novel proteoforms, and detection of unknown biochemical pathways. The use of C-terminal analysis has been limited so far mainly because of the technical challenge of dealing with the intrinsically low chemical reactivity of carboxyl groups. However, in recent years technological advances in mass spectrometry have made it possible to develop more efficient analytical techniques targeting C-termini.

Studying the C-terminomics of plant abiotic stress responses could be a way to push forward this domain. C-terminal analysis techniques and principles developed in recent years will be reviewed and discussed here.
Beans are one of the leading legume plants in the world. Beans are cultivated for fresh pods or dry seeds in the field and for fresh pods in greenhouses in winter. Chilling (temperatures below 10 °C) and freezing stress (temperatures below 0 °C) are considered to be the most important stress factors limiting the production of the plant during generations in the spring and in autumn. In this study, 55 genotypes selected for their cold tolerance from growth chamber and field trials were studied for molecular characterization.

The molecular characterization of a core collection of 55 landrace and cultivars was determined by the Sequence Related Amplified Polymorphism (SRAP). Molecular characterization studies were performed using 27 ISSR primers to generate the dominant data. In total, 211 DNA fragments were scored, 109 of which were polymorphic.

Future field and greenhouse adaptation studies using selected genetic resources will provide safer crops in early spring and late autumn and winter with the development of fresh bean varieties tolerant to chilling and freezing stress.

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Oil quality of sunflower (Helianthus annuus L.) is determined by mostly fatty acid contents in the seeds. Normally sunflower has higher linoleic acid which is more suitable for cooking oil but it uses commonly for frying oil in many part of the world especially Eastern Europe, Asia, etc. On the other hand, oleic acid among fatty acids is known for higher oil quality and is more suitable for frying with giving also longer shelf life in oilseeds such as olives, canola etc. Likewise, higher oleic genes were discovered in Pervenent mutations in sunflower and transferred into cultivated ones. The use of higher and mid oleic type sunflower oil increase recently because of giving to consumer higher quality oil. Therefore, higher and mid oleic sunflower production increase year by year in US, Argentina, France, Spain and other countries. The selection of seed traits such as oleic acid content is very hard process because it needs to wait until harvesting and oleic acid content of seeds could be determined with Gas Chromatography (GC) after threshing and analyzing of seed. On the other hand, oleic acid genes were not highly inherited and influence from environmental conditions such as night temperatures during the grain filling period in sunflower. New molecular tools such as molecular markers will help both for efficient selection and also to help accelerating and shorten breeding programs. Therefore, molecular markers containing high oleic acid make possible to obtain faster and more consistent results on selection processes in plant breeding program as well as including reducing the workers and obtaining more precise selection. The screening process of sunflower genotypes have been performed with 9 markers as total which were 3 SSR and 6 INDEL markers from the FAD2 gene section that were detected to be related to high oleic acid in this study. All the samples has been compared with analysis in terms of oil acids in GC device in order to detect if the markers have done the selection correctly or not. As a result of this study, 4 markers were determined properly as (N1-3F)/(N2-1R) HO, F4-R1, F4-R1 and F4-R1 to select high oleic acid trait having homozygous oleic gene. As results, the screening of sunflower genotypes for high oleic content will be possible precisely for further generations utilizing these four molecular markers in sunflower breeding programs.
Guard cells shrink and close stomatal pores when air humidity decreases (i.e. when the difference between the vapor pressures of leaf and atmosphere [VPD] increases). The role of abscisic acid (ABA) in VPD-induced stomatal closure has been studied using ABA-related mutants that respond to VPD in some studies and not in others. The importance of ABA biosynthesis in guard cells versus vasculature for whole-plant stomatal regulation is unclear as well. Here, we show that Arabidopsis (Arabidopsis thaliana) lines carrying mutations in different steps of ABA biosynthesis as well as pea (Pisum sativum) wilty and tomato (Solanum lycopersicum) flacca ABA-deficient mutants had higher stomatal conductance compared with wild-type plants. To characterize the role of ABA production in different cells, we generated transgenic plants where ABA biosynthesis was rescued in guard cells or phloem companion cells of an ABA-deficient mutant. In both cases, the whole-plant stomatal conductance, stunted growth phenotype, and leaf ABA level were restored to wild-type values, pointing to the redundancy of ABA sources and to the effectiveness of leaf ABA transport. All ABA-deficient lines closed their stomata rapidly and extensively in response to high VPD, whereas plants with mutated protein kinase OST1 showed stunted VPD-induced responses. Another strongly ABA-insensitive mutant, defective in the six ABA PYR/RCAR receptors, responded to changes in VPD in both directions strongly and symmetrically, indicating that its VPD-induced closure could be passive hydraulic. We discuss that both the VPD-induced passive hydraulic stomatal closure and the stomatal VPD regulation of ABA-deficient mutants may be conditional on the initial pretreatment stomatal conductance.
Comparative analysis of genetic diversity among cultivated wheat (Triticum aestivum L.) and its related species using microsatellite markers.

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One of the prerequisites for further improvement of wheat is the assessment of genetic variability of wild relatives, for the identification of desirable traits that can be used for the genetic enhancement of elite material. The aim of this study was to analyse the polymorphism of 15 microsatellite loci in the collection comprising of 40 genotypes of bread wheat (Triticum aestivum L.), 32 genotypes belonging to other species within Triticum genus, and 3 genotypes from Aegilops genus. The results showed high level of genetic diversity present in the collections of cultivated wheat and its relatives. In the collection of bread wheat genotypes, 119 alleles were detected, while in related species 157 alleles were identified. The average gene diversity of 0.68 and 0.81 were found in cultivated wheat and relatives, respectively. The average PIC value in bread wheat was 0.65, and in related species was 0.79. Different dominant alleles were identified in the collections of bread wheat and related species at 9 out of 15 examined loci. Higher number of alleles in the collection of related species was found at 10 loci, while at three loci (Xbarc12, Xgwm284 and Xwmc457) higher number of alleles was found in bread wheat genotypes. The two loci (Xgwm495 and Xgwm3) had the same number of alleles in both groups of genotypes. Analyses of individual genomes indicated that only the A genome had similar genetic variability indicators in the both groups of genotypes, while genetic diversities of the B and D genomes in the cultivated wheat were significantly reduced in relation to the other species. All analysed parameters of genetic diversity showed significantly higher level of polymorphism present in the wild relatives than in the cultivated bread wheat, indicating a huge potential of using wild related species as a source of new genetic variability for further improvement of agronomically important traits in bread wheat.
56 - High-throughput multi-sensor phenomics analysis of drought tolerance in tomato plants with different level of glycinebetaine.

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Plants are frequently exposed to various abiotic stresses during their life cycles and drought is dominant growth reduced factor. One of mechanisms of the plant drought tolerance is accumulation of compatible solutes. Using high-throughput PlantScreen™ phenotyping platform with multi-sensor imaging facilities (FLUO, RGB, VNIR and SWIR imaging) have been studied growth dynamics and functional physiology in wild-type (WT) tomato plants (Lycopersicon esculentum, cv. Moneymaker) and their four GB-accumulating lines growing in soil under precise water supply control. During this study, the methodologies for multi-sensor phenomics data collection of tomato plants under drought and image analysis were tested and verified. Finally, data analysis showed that GB-accumulating lines were different in some shoot geometrical traits (total plant area, compactness and SOL) and growth rate in well-watered conditions compared to WT. Hyperspectral VNIR reflectance analysis reveals a different ability of GB-lines to synthesize leaf pigments (chlorophyll, carotenoids and anthocyanins). Under drought conditions, WT plants significantly reduced growth rate and plant area compared to GB-accumulating lines. Higher capacity of drought tolerance in GB-accumulating tomato lines under water shortage was manifested by maintenance of photochemical PSII efficiency, higher NPQ and less reduction of growth rate. Hyperspectral SWIR reflectance imaging reveals a higher water content in GB-lines. The analysis contributed to our knowledge of the phenotype formation structural and functional traits in tomato plants differing in accumulation of GB. Moreover, multi-sensor phenomics analysis enables the description of drought tolerance formation and next functional relationships between GB accumulation, photosynthesis and growth maintenance under drought. The study is supported by APVV-15-0721, APVV-15-0562, VEGA-1/0923/16, VEGA-1/0831/17, and UE H2020 project No 731013 (EPPN2020).
To explore and understand the mechanisms of plant adaptation to environmental stresses, it is essential to determine patterns of protein expression, to identify the stress proteins and to match them to the specific functions in metabolic pathways. The main purpose of this study has been to analyze and compare changes occurring in the proteome of leaves of seedlings of grapevine varieties characterized by different degrees of tolerance to the prolonged exposure to cold. The isolated proteins were separated by the two-dimensional electrophoresis (2 DE) technique and identified with the tandem mass spectrometry MALDI TOF/TOF type. The analysis of the proteome of grapevine leaves enabled us to detect many proteins whose synthesis changed under the influence of the applied stressors. It was demonstrated that the persistent exposure to cold caused changes in the synthesis of key enzymes of the glycolytic pathway and proteins which are an essential component of photosystem II. It was shown that many of the identified proteins tended to achieve a similar level of accumulation under cold stress conditions in leaves of a variety sensitive to cold and another one tolerant to this stressor. However, a few proteins presented specific accumulation profiles in leaves of the cultivars with different tolerance to cold or else a change in their accumulation during cold stress was observed in leaves of one of the tested varieties. These proteins can play a role of potential biomarkers of the cold sensitivity of grapevine.
Drought is a limiting factor in crop productivity, and roots are the first organs that respond to drought in the soil. At present, it is difficult to study the growth and structure of the root system, especially in field conditions. Classical approaches to analyzing root traits in field conditions are still considered standard techniques. Over the past decade, new modern and nondestructive methods have been introduced into root research. These approaches use advances in automation and computer-aided analysis. In the experiment, we focused on the effect of drought on modulation of root architecture, where four genotypes of soybean (Glycine max L. Merrill), all origin from China were used. Plant roots were manually extracted from the pots at the end of vegetation growth (BBCH 49) and root phenotype traits were measured with RGB imaging unit of the PlantScreenTM system. The length of the root system and the counting of nodules was manually evaluated by using software Image-J. Our findings lead to the conclusion that the drought led to a reduction of absorption area of root and a significant increase in root growth, rather to length than thickness. Finally, we found a close correlation between automatic and manual counting the number of nodules. RGB imaging analysis is enables better characterization of root architecture of plants affected with drought. This work is supported by APVV-15-0721, APVV-15-0562, VEGA-1/0923/16, VEGA-1/0831/17, and UE H2020 project No 731013 (EPPN2020).
Tobacco plants with the desA gene under the control of the CBF1 promoter were cultured in vivo and tested for resistance to hypothermic stress

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One of the mechanisms to increase the resistance of plants to abiotic stress is to increase the proportion of unsaturated fatty acids in the membrane lipids. Desaturases are enzymes that promote the formation of double bonds in fatty acids and thus transform them from saturated to unsaturated. The desA gene encoding Δ12-acyl-lipid desaturase of cyanobacteria Synechocystis sp. PCC 6803 was used in work. This gene was in one frame reading with the gene of the reporter protein of the thermostable lichenase licBM3 bacterium Clostridium thermocelum. Transgenes can affect the physiological functions of plants, both positively and negatively, therefore, it is necessary to reduce their negative impact to a minimum, regulating their expression with the help of specific promoters. The cold-induced promoter CBF1, the sequence of which was cloned from the genome of the Arabidopsis thaliana plant, was used in this case. Transformation was carried out by Agrobacterium tumefaciens-mediated method. These plants were cultured under in vivo conditions for further studies. Controls were made of wild-type Nicotiana tabacum plants and N. tabacum containing gfp::licBM3 gen. The level of damage to the investigated plants was checked after stress (0°C - 20 min, -5°C - 80 min). The studied plants showed a lower accumulation of malonic dialdehyde, a lower level of electrolyte loss and increased activity of the enzyme superoxide dismutase compared to control plants.
Different morpho-physiological traits have been proposed as key traits associated with grain yield potential of wheat. Considering that methods for assessing morpho-physiological traits are laborious and expensive, phenotyping via remote and proximal sensing techniques could contribute to improvement in wheat breeding programs. The NDVI (Normalized Difference Vegetation Index) represent one of the most promising tools for application in field phenotyping with potential to provide complex information on different morpho-physiological traits of wheat. The objective of this study was to analyze the use of different NDVIs derived from field reflectance measurements to estimate grain yield, plant height, aboveground biomass, total leaf chlorophyll and nitrogen content for the contrasting wheat cultivars. The NDVI was measured using an active hand-held sensor GreenSeeker (NTech Industries Inc., Ukiah, California, USA) and hyperspectral camera (Ximea Corp., Lakewood, CO USA) at four growth stages of wheat: full flowering, medium milk, early dough and fully ripe stage of wheat. Based on two-band combinations between red (600 - 700 nm) or far-red (700 - 750 nm) and near-infrared (756 - 955 nm) regions, 66 different hyperspectral NDVIs were calculated. Significant and positive correlations (higher than 0.6 and significant at p < 0.05) were found between the specific hyperspectral NDVIs and morpho-physiological traits, but varied with growing stages and genotypes. Furthermore, hyperspectral NDVIs provided an overall better estimate than GreenSeeker sensor since they provided additional spectral band combinations for NDVI, exclusively sensitive to targeted morpho-physiological traits of wheat. This study give promising results which can be used as a basis for development and improvement sensing devices based on wider range of wavelengths which could lead to achievement real-time information in monitoring key traits associated with grain yield potential of wheat.
Field-based phenotyping of growth and biomass yield of forage crops, in the context of research and breeding, involves monitoring a huge number of replicated plots over several seasons. In these extensive trials growth is usually determined using visual scores, in combination with destructive measurements of biomass yield, five times per year. Because visual scores are time-consuming they are not performed frequently. In addition, visual scores are assigned to a complete plot, ignoring possible spatial heterogeneity inside the plot. Therefore, current methodology suffers from low temporal and spatial resolution. In addition, visual scoring systems are prone to biases due to subjectivity of the evaluator. Here, we present a methodology based on the use of visual images captured using a drone that allows determination of canopy height and estimation of plant growth throughout the season at high spatio-temporal resolution.

During the growing season 2017, a drone equipped with a visual camera was used to perform a total of 22 flights over a field trial consisting of 1350 micro-plots of perennial ryegrass (Lolium perenne) located in Merelbeke, Belgium. The field trial is part of GrassLandscape, a FACCE-JPI ERA-NET+ project, and comprises 500 accessions that together represent the natural diversity of the species across Europe. The flights covered the main growing period of perennial ryegrass in Belgium, from 25/04/2017 until 03/10/2017. Orthophotos were generated and processed and the structure from motion (SfM) technique was used to build digital elevation and digital surface models (DEM and DSM, respectively). The orthophotos had an RMSE in the XY plane of approx. 0.7 cm. DEMs had an RMSE in the Z plane of approx. 1.2 cm. For calibration purposes, frequent destructive measurements of biomass were carried out. An overview of the first results regarding correlation between UAV-estimated data and biomass data gathered using destructive methods will be presented.
Composition of essential oil of Thymus x citriodorus in relation to meteorological factors

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Essential oils and/or their chemical compounds are used in food industry as food flavouring and natural antioxidants, in pharmaceutical industry for manufacturing of pharmaceuticals, in cosmetic and perfume industry as aromatizers. Lemon thyme (Thymus x citriodorus) is perennial subshrub with rose-lemon-like flavor. Acyclic monoterpene alcohol geraniol, the main compound of essential oil of T. x citriodorus, is a fragrance ingredient of cosmetic products and characterized by wide spectrum of antibacterial activity. In T. x citriodorus, geraniol always co-exists with three biogenetically related compounds: nerol, geranial and neral. The aim of this work was to establish the effect of meteorological factors on percentage of geraniol, nerol, geranial and neral in essential oil of T. x citriodorus.

T. x citriodorus was grown in the field collection of the Nature Research Centre (Vilnius, Lithuania) three year in an open ground. The aerial parts were annually collected at the full flowering stage and dried at room temperature. The essential oils of leaves-inflorescences were isolated by hydrodistillation and analyzed by GC/MS. The meteorological data were obtained from the closest station of meteorology of Lithuanian Hydrometeorological Service under the Ministry of Environment.

Temperature (T), rainfall (R), sunshine duration and photosynthetically active solar radiation negatively, sum of R from April to June - positively influenced on amount of essential oil of T. x citriodorus. Geraniol was the richest compound of essential oil of T. x citriodorus during investigation years: it amounted 1.2 to 3 time higher percentage than nerol, geranial and neral. Variation of percentage of geranial was the lowest during 2015–2017, while the variation of other investigated compounds was very similar. Sums of averages of monthly T, Sd and PAR from April to June negatively, R – positively influenced on percentage of geraniol in essential oil of T. x citriodorus.
One of the major concerns of modern agriculture in Armenia is the conservation and utilization of valuable genetic resources of agricultural plants. Grape breeding activities are mainly focused on the development of new cultivars which show quality characteristics quite comparable to traditional cultivars but exhibit additionally a high degree of resistance, mainly against the Downy mildew. The available grape biodiversity in Armenia and its multidisciplinary study still allow us to identify valuable genetic resources, possibly with resistance to pathogens and abiotic stresses. Breeding for resistance to this disease is crucial to avoid extensive fungicide applications that are costly, labour intensive and may have detrimental effects on the environment. Thus, the search for alternative methods to control grapevine downy mildew is of paramount importance for viticulture. Resistance to downy mildew can be evaluated using field evaluations, greenhouse based screening methods or laboratory-based techniques. Of the methods reported, the wide use of the leaf disc method is approved as the most reliable technique used to assess downy mildew resistance in the laboratory.

Phenotypic evaluation of thirty Armenian grape genotypes by leaf disc method and molecular characterization by DNA-based molecular markers related to resistance genes of downy mildew were realized. The grape cultivars with different level of resistance to downy mildew were described and further analysis of promising genotypes with the involvement of additional markers will be done. The results of the realized research will guide long-term breeding strategies and the development of new cultivars targeted to increase the quantity and value of grapes consumed. We expect that Armenian indigenous varieties and wild Vitis can be an essential germplasm resource for disease resistance breeding, and contain especially useful breeding material for improving the disease resistance of grapes.
Plant genetic resources are one of the most important natural resources for an agricultural country such as Serbia. Institute of Field and Vegetable Crops (IFVCNS), Novi Sad (NS), maintains the collections of major and minor oil crops, including safflower and false flax, that are used in breeding. Safflower (Carthamus tinctorius L.) that belongs to the Asteraceae (Compositae) family, is a thistle-like drought-resistant annual plant that is grown commercially for the production of oil and birdseed. False flax (Camelina sativa (L.) Crantz) is an oil crop from Brassicaceae family, which has become particularly interesting in recent years due to its diverse use and modest growing and cultivation requirements.

NS safflower collection consist of genotypes of different geographical origin (Ukraine, Italy, Turkey), while false flax collection comprises accessions exchanged between Universität für Bodenkultur Wien (BOKU) and IFVCNS, the participating institutions in the Serbian-Austrian bilateral cooperation project ?Introducing Camelina - a sustainable future oil crop? (2016-2017). Phenotypic observations of safflower genotypes during two growing seasons revealed that analyzed genotypes differ in flower color (yellow, orange, red), presence of spikes and in seed oil and protein content. Oil and protein content differed between years and genotypes, indicating large influence of genotype and environmental conditions on variations of these quantitative traits that are negatively correlated. False flax accessions were tested in the trial set up as lattice design and sown at two locations (Tulln, Austria and Rimski ?an?evi, Serbia). Oil and protein content analyses showed the differences between the two locations. Owing to the importance of these two minor oil crops and increase of their production as resilient crops, they were involved in our breeding programs and the cultivar registrations are in progress for the first time in Serbia.
65 -Cellular and carpological phenotyping for improvement of local viticulture in Armenia.

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Armenia is one of the homelands of grapes and wine. Phenotypic and genotypic characterization of grapevine gene pool is important for the detection of tolerant genotypes, maintenance and sustainable use of grape biodiversity. Future progress in crop breeding requires a new emphasis in phenotyping at plant and cellular level. Reproductive cell biology and agrobiological traits of crops are related to the origin and genetic characteristics of varieties, gene expression and phenotype development in different environmental conditions.

Phenotyping of eight Armenian grapevine varieties of different origin grown at the Nalbandyan collection (Armavir province, Armenia) was realized by cytoembryological and agrobiological traits. Flowers were treated by common paraplast-embedding and sectioning techniques with double staining by Mayer (H&E). The permanent slides were analyzed by Motic M-10 digital microscope. Pollen viability was determined using aceto-carmine method. Morphometric analysis of ripe berries was realized on digital photos using ImageJ software according to OIV Descriptors.

High levels of reproductive sterility strongly affect the quantity, size and quality of berries. In the ovaries instead of 4 ovules per flower in average only from 1.4 to 2.8 ovules with eight cells of embryo sacs were developed irrespective of their origin. At the same time our results demonstrate high level of pollen viability varied from 87.5% to 99.4% in ancient and from 59.6% to 91.2% in new bred varieties.

Morphometric analysis revealed the formation of berries with average weight from 1.3 to 5.6 g and average sizes from 13.9 mm to 25.0 mm. No significant differences in berry characteristics between ancient and new bred cultivars were revealed. Our data can be useful for improvement of grape quality and saving costs and time in targeted cross-breeding programs.

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Seed vigor is a complex trait relying on various physiological processes and metabolic pathways globally ensuring an optimal germination rate and an efficient seedling establishment, essential features for plant propagation and crop production. A key component of seed vigor is represented by the ability to preserve genome and cellular structures from oxidative damage naturally occurring during seed imbibition and dry storage, as well as upon the exposure to biotic and abiotic stress (Waterworth et al. 2016, Proceeding of the National Academy of Sciences U.S.A., 113, 9647-9652; Pagano et al. 2017, Frontiers in Plant Science 8, 1972). The possibility of relevant outcomes for ecology and agriculture has lead seed companies and plant biotechnologist to improve the current knowledge concerning the role of DNA Damage Response in determining seed vigor (Paparella et al. 2015, Plant Cell Reports 34, 1281-1293). The present work explores for the first time the signatures of genotoxic stress on seed metabolome.

Seeds of the model legume Medicago truncatula has been imbibed in presence/absence of rage of stress agents throughout seed imbibition. A preliminary biometric evaluation of their effects showed an altered phenotype with delayed and decreased germination rates along with impaired seedling growth. Single Cell Gel Electrophoresis (Comet assay) performed on the radicle protrusions revealed increased DNA damage in comparison to water-imbibed seeds while qRT-PCR (Quantitative Real-Time PCR) indicated a significant up-regulation of antioxidant and BER (Base Excision Repair) genes throughout seed imbibition. Finally, a non-targeted metabolomic profiling highlighted a chemically and functionally heterogeneous subset of metabolites representing putative signatures of repair response in seeds.
Photosynthesis is one of the most important plant traits affecting on plant growth and productivity under optimal and stress conditions. Diurnal variation in photosynthesis characteristics challenges the characterization of specific genotypes and their response to different environmental conditions. To overcome this challenge, an open gas-exchange measuring system with self-clamping leaf chambers has been developed to ensure simultaneous monitoring of photosynthesis in leaves of intact plants. This technique was implemented in a commercial device, PTM-48A Photosynthesis Monitor. This system was used in greenhouse and under control conditions in growth chamber.

We found that this equipment gives reproducible measurements by using in growth chamber where constant CO2 concentration were maintain. However, in greenhouses with CO2 enrichment, variability of CO2 concentration in the air negatively affected the reproducibility of measured values. This challenge was overcome by parallel replicated measurements, which helped to select the reliable values of photosynthesis. Other advantages and challenges of photosynthetic monitoring system are discussed in the context to plant rapid response to continuously changing environments and diurnal fluctuation of photosynthesis.
Sugar beet (Beta vulgaris L.) has been mainly selected for sugar production. High yields have been achieved by the use of irrigation and this crop is described as being able to cope well with mild salinity (40-120mM NaCl). However, during seed germination sugar beet is considered a salinity sensitive crop and soil salinity should not exceed 30mM NaCl. Wild beets, ancestor species of sugar beet, are naturally able to germinate and grow in saline environments. We evaluated the germination and early seedling development of three Portuguese ecotypes of wild beet: Comporta (CMP); Oeiras (OEI); Vaiamonte (VMT); and one sugar beet cultivar (Isella). Two distinct effects of salinity are revealed: in the ability to initiate radicle emergence, VMT outperforms the other ecotypes; in the ability to secure seedling growth, CMP exhibits the best performance, i.e. the highest relative salinity tolerance index, despite reduced seedling growth and biomass. Since the abundance of membrane proteins in the cotyledons was negatively affected by salinity, the hypothesis was raised that the functionality of the membranes, including the photochemical multiprotein complexes, was compromised. To test this hypothesis, a blue-native two-dimensional electrophoresis was applied. The proteomic evaluation shows that the trade-off of seedling growth under saline environments is a lower photosynthetic capacity of cotyledons, the main photosynthetic organ. Salinity induced loss of functionality of the membrane multiprotein complexes, mainly due to lower abundance of ATP synthase subunits and of photosystem I components. In hypocotyls, photosynthesis does not seem to be a major process, but protein synthesis and trafficking can have relevant roles. These processes require energy consumption, which is supported by an increase in ATP synthase abundance observed in our data.
Augmented reality glasses for monitoring plant health
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Augmented reality (AR) is a live direct or indirect view of a physical, real-world environment whose elements are
70 - Comparative Study of Rhizobial and Mycorrhizal Symbionts on Yield and Seed Quality of Pea Plants upon Stress Conditions via an Integrative Phenotypic-Metabolomic-Proteomic Assessment.

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Crops yield and quality enhancement is an inevitable priority on global scale for food security and sustainable agriculture upon climate change. Another key component is the improvement of the plants resistance levels against biotic stress. Recently, it has been demonstrated that soil-microsymbionts play an important role for enhanced stress tolerance. Hence, a global quest to study the impacts of plant growth promoting microsymbionts on crop plants based on integrated phenomics-omics approaches are coming progressively into focus. A major pathogen of pea is the fungus Didymella pinodes leading to significant losses in grain yield every year. In our current study, we assess and compare the effects of Rhizobium leguminosarum bv. viceae (Rlv) and Glomus mosseae fungi on whole above-belowground parts of Pisum sativum of two different resistance levels with a major focus on seed quality and yield upon stress caused by D. pinodes. Although both microsymbionts improved significantly productivity of pea plants through increased yield components, rhizobial symbiosis revealed higher impact on seed physical properties as well as root and seed dry weights in comparison with mycorrhizal symbiosis. However, some plant growth parameters including pod number and size and seed number per pod were largely affected by mycorrhizal compared to rhizobial symbiosis. Hence both, Rhizobium and mycorrhiza symbioses lead to a notable reduction of disease severity upon pathogen infection, however, the rhizobial effect was stronger. This was caused by the microsymbiont induced changes in seed metabolome and proteome of the analyzed pea cultivars. In addition to late embryogenesis abundant proteins, cell wall and redox related proteins were notably induced. Also, seed metabolites, especially flavonoids, phytohormones, lipids and other organic compounds with significant promoting impact on symbiosis establishment with pea plants and effective against pathogen infection are being presented.
Iron deficiency chlorosis (IDC) is a serious problem affecting legumes grown in calcareous soils. Apart from the obvious effects of IDC on plant yield and quality, climate change is intensifying the severity of this nutritional problem, leading to a need in the discovery of sustainable strategies to improve iron uptake by plants. The use of plant growth-promoting bacteria (PGPB) could be one of such strategies. However, before implementation, it is necessary to conduct a combination of extensive plant and PBGB phenotyping to identify the most suitable combination of PBGB for each specific cultivar. The first goal of this study was thus to phenotype a collection of PBGB available at CBQF for three tests related with iron nutrition: qualitative analysis of organic acid production, ability to reduce iron (III) and growth under different pH values, evaluated as percent inhibition. The second was to isolate and identify novel PBGB which are naturally associated with legumes, and expand the collection that can be used for these purposes. Lastly, soybean plants were subjected to IDC prone soil conditions and the two most promising bacterial strains (Sphingobium fuliginis and Pseudomonas jessenii), alone or in combination, were tested as IDC correctors. A series of plant phenotypic parameters related with IDC development were then evaluated, such as SPAD values, root iron reductase activity, plant dry weight and mineral content, showing the applicability of these bacterial strains as novel potential agents for IDC correction in the context of climate change.
Orobanche crenata is a root parasitic weed constraining legume cultivation in Mediterranean Basin and Neat East. Other species such as O. foetida and O. aegyptiaca can also be of local importance. Breeding for resistance is possible, but is hampered by the lack of sufficient levels of resistance, the complexity of its inheritance and the unreliability of available screening methods. Recent achievements in the identification of resistance levels and their deployment in breeding programs will be presented and critically discussed.

Significant progress has been made in pea (Pisum sativum) and faba bean (Vicia faba) breeding by accumulating the available quantitative resistance by crossing and selections, resulting in the successful release of resistant cultivars. Proper phenotyping at cellular and plant level allowed the identification of mechanisms of resistance operative, postulating gene/QTLs and markers that might accelerate resistance breeding. Resistance have also been identified in common vetch (V. sativa), chickpea (Cicer arietinum), lentil (Lens culinaris), and grasspea (Lathyrus sativus), as well as in their wild relatives.

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Impact of water deficit on growth and yield components of two garlic (Allium sativum L.) cultivars at different crop stages.
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Expected droughts in the context of climate change are likely to lead to modifications in the soil water availability throughout crop stages. In the search for drought tolerant varieties, inter-cultivar variability in response to water availability needs to be screened throughout the crop cycle. In this study, key traits such as bulb weight, root weight and relative growth rate in pseudo-stem diameter (RGRps) in garlic were studied. We selected two common Spanish cultivars: the commercial cultivar ?Gardacho? (GAR) and the landrance ?Morado de Pedroneras? (PED). The impact of water availability depending on the crop stage, vegetative or bulbing phase, was evaluated by application of four water treatments: a) Water deficit in both crop stages (WW); b) Water deficit on vegetative phase (SW); c) Water deficit on bulbing phase (WS) and d) Well watered in both crop stages (WW). Results confirm that water deficit applied on different crop stages resulted in significant differences in the studied traits. Bulb weight was especially affected when water deficit was applied in the bulbing phase in both cultivars. Nonetheless, while bulb weight of GAR was affected by SS and SW in comparison to WW, that of PED recovered to WW values at SW. Furthermore, PED presented significantly heavier bulbs in this water treatment than GAR. Both factors (i.e. cultivar and water availability) had a significant effect on root weight, with notably weightier roots on GAR. Additionally, GAR and PED responses to RGRps varied to these four water treatments. Generally, GAR presented higher RGRps in every treatment but this effect was notably accentuated on SW and WS. Interestingly, we found that differences in growth and yield between cultivars depended on the vegetative phase in which water deficit was applied. Thus, we conclude that selecting the appropriate cultivar and period for irrigation should be key aspects to be considered for maximizing garlic crop yields in future climate scenarios.
74 - SSR-based association mapping of drought tolerance in F2 segregated population derived from reciprocal cross of Sagittario x M6 Sagittario derived drought tolerant mutant(s).

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Drought is globally a major constraint for crop production. Development of improved crop cultivars with drought tolerance is critical for sustainable crop production. Induced mutations change in plant architecture play a significant role in adaptation to stress conditions. Therefore, mutants are valuable germplasm resources in crop improvement programs. This study has dealt with mapping QTLs for drought tolerance in F2 segregated population derived from reciprocal cross between Sagittario and M6 Sagittario derived drought tolerant mutant(s). A total of 100 segregated individuals were phenotyped at optimal conditions and in response to drought treatment. Genotypic variations in population were screened with 178 SSR markers. Population structure was determined with STRUCTURE 2.3.3. Phenotypic and genotypic data were associated using TASSEL 2.1 to find associated markers with agronomic traits. The identified markers can be used in marker-assisted selection mediated breeding methods.
75 - WineClimAdapt - Selection and characterization of grape varieties best suited to climate change scenarios foreseen for the near future.

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Grapevines are among the plant species with greater genetic variability, reflected in the several thousand of varieties grown worldwide and this variability is one important resource for adaptation to climate scenarios. In this work we present the methodologies applied in a 5 years research project, WineClimAdapt, started in 2018. The main goals are to evaluate the resistance of 200 varieties to abiotic stress, namely thermal and drought stress, applying recent tools for a time integrated characterization at field and whole plant level and, in a second phase, to evaluate the agronomic and enological behavior. Two ampelographic fields with contrasting environments, in dry and very hot conditions (Alentejo) and in temperate conditions (Estremadura) will be used. These fields will be characterized according to: (i) crop phenology - used to examine the cultivars phenological dynamics using phenological timing and growth intervals in order to evaluate cultivars adaptability to climate change; (ii) stable isotope signatures - applied at plant level to develop an
The consequence of elevated carbon dioxide (eCO2) on plant responses is an important topic and has been the subject of several scientific research. This study was conducted to determine the effect of eCO2 on 18 bean and soybean genotypes. They were grown at indoor controlled-environment chambers under eCO2 of 800 ppm and ambient CO2 of 400 ppm conditions. Our results showed that in general there was a large variability of response within cultivars and between species. Across all genotypes, the percent change at eCO2 ranged from -67 to +50% in pods per plant, -6 to +42% for leaf chlorophyll concentrations, -67 to +70% for seeds per plant, -35 to +85% for 100-seed weight, -72 to +35% in grain yield, -70 to +30% in aboveground dry weight, and -32 to +16% in height for soybean genotypes.

In terms of minerals content, the [Zn] ranged from -17 to +20%, [Mn] from -53 to +44%, [Fe] from -29 to +13%, [P] from -31% to +43, [Mg] from -1 to +40%, [Ca] from -26 to +69%, and [K] from -12 to +15% percent change at eCO2 for soybean genotypes.

Regarding bean, the percent change at eCO2 ranged from -46 to +80% in terms of pods per plant, the non-destructive measurement of leaf chlorophyll concentrations varied from -27 to +21%, the seeds per plant from -46 to +81%, the 100-seed weight from -46 to +4%, -64 to +42% in terms of grain yield, -32 to +50% for aboveground dry weight, and from -21 to +49% for height. For bean, the [Zn] ranged from -23 to +10%, [Mn] from -47 to +26%, [Fe] from -30 to +22%, [P] from -12% to +14, [Mg] from -12 to +14%, [Ca] from -21 to +36%, and [K] from -22 to +16%. Seed nitrogen concentration also ranged from -5 to +11% and -10 to +9% for bean and soybean genotypes, respectively. Our results provide significant intraspecific differences among individuals from both species tested in terms of CO2 enrichment and show that there is enough cultivar variability to select cultivars with better performance.
Global atmospheric CO2 concentration is projected to reach 550-600 umol mol⁻¹ by the middle of this century. This increase may improve the photosynthetic efficiency of plants, which generally leads to an increasing supply of photoassimilates, and thus biomass and yield. The response of plants to elevated CO2 (eCO2) differs from one species to another and even within the same species. Free-air CO2 enrichment (FACE) technology is currently regarded as the most realistic and natural CO2 exposure system. A FACE experiment was carried out at Campus Klein Altendorf (Germany) to investigate the effects of eCO2 on 14 US and European bean genotypes. The different genotypes were grown at either 400 umol mol⁻¹ or at 600 umol mol⁻¹ CO2 concentration under FACE experimental facility. Across all genotypes eCO2 increased dry weight (+37%), height (+15%), leaf area (+37%), leaf chlorophyll concentration (+3%), normalized difference vegetation index, an index of plant greenness (+2%). These parameters were measured between 53-68 days after sowing. Additionally, also increased seeds per plant (+11%), grain yield (+35%) and 100-seed weight (+18%). At eCO2 an increase in net photosynthetic rate was observed, as well as water use efficiency and stomatal conductance of upper most fully-expanded leaves. The seed nitrogen concentration decreased (-1%). Our results provide robust estimates of plant responses to CO2 enrichment and demonstrate important differences among individual taxa.
78 - PtaUGT72E1, a glycosyltransferase involved in the regulation of lignification in poplar.

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In plants, UDP-glycosyltransferases (UGT) are a superfamily of enzymes catalysing the transfer of glycosyl groups to a wide range of molecules like hormones, xenobiotics and secondary metabolites, ... These enzymes are crucial in many environmental adaptation mechanisms. In Arabidopsis thaliana, the UGT72E family was shown to function with monolignols, deriving from the phenylpropanoid pathway. Monolignols are the precursors of many essential compounds for plant growth and development, like intracellular di-, tri- or oligolignols which are considered to have plant defense properties. In the cell wall, they can polymerised to form lignin. It is a complex polymer, which has a role in plant support and allows, thanks to its hydrophobic character, the conduction of water and solutes in the xylem. Currently, our knowledge on the regulation mechanisms of monolignol polymerization into lignin and lignans remains limited. The glycosylation of monolignols and their storage in the vacuole are considered as central mechanisms for monolignol homeostasis. The purpose of this work is to gain a better knowledge on the role of UGT72E in woody plants, producing a large amount of lignin, by characterizing PtaUGT72E1 in Populus tremula x P. alba (clone INRA 717-1B4). One of the strategies chosen is the phenotyping of transgenic poplar over-expressing and down-regulating PtaUGT72E1. A particular focus was made on stem lignification. Two methods were chosen to analyse this aspect: fluorescent analysis of safranine staining and Raman spectroscopy. Both methods confirm the alteration of lignin deposition in the xylem cellular wall in transgenic lines: a decrease of lignification was recorded in the over-expressing lines and an increase of lignification in the down-regulating lines. These results highlight a possible role for PtaUGT72E1 in the regulation of the lignification of vascular tissues in poplar.

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Breeding for drought tolerant crop varieties have mainly relied on genetic selection based on aboveground observation of canopy responses to drought. A potential lies within non-destructive studies of root development and rooting depth to identify deep rooting varieties that may better utilize deep soil resources. Previously, root studies have been cumbersome and non-destructive assessment utterly challenging. A semi-field screening facility called RadiMax has been established at the University of Copenhagen, Denmark. Here roots are studied through 150 transparent minirhizotrones per unit (40*10 metre) in a total of 4 separate units, using camera system down to 2.8 meters soil depth. Direct root phenotyping is combined with a water stress gradient across each unit created by a multi depth sub-irrigation system and rain-out shelters. As water stress gradient should affect canopy growth, an additional aspect is to non-destructively measure water stress responses on canopy using Unmanned Aerial Systems (UAS). Initial studies of canopy stress were made in 2017 with imaging by color (RGB) camera, multispectral camera and a thermal camera on an UAS in a multisensory setup. The results revealed that a drought stress gradient was visible in the facility in barley varieties at anthesis, albeit only in the thermal images. Here canopy of the barley varieties where cooler near the edge of the pit, and warmer towards the middle of the pit, where also the depth to sub-irrigation is largest. Furthermore, studies of roots showed genotypic differences in rooting depth between varieties. The setup enables the possibility of comparing rooting depth, measured canopy response to drought and aerial imaging. In 2018, this multiscale approach of drought studies and root studies will be intensified on genotypes of winter wheat and potatoes. Measurements from UAS thermal, RGB and Multispectral imaging will be validated by canopy measurements and compared to rooting depth.
Phenotyping of specific response to salt stress of different pigmented wheat genotypes to determine salt tolerant varieties.

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The aim of this work is to study salt stress effect on the development adaptive reaction and growth parameters of different pigmented wheat genotypes to estimate parameters which can help to choose salt-tolerant wheat cultivar. The different pigmented wheat genotypes has been characterized by different anthocyanins content which partly can affect development of adaptive reaction on increasing of salinity stress. On the early stage of treatment with salt for lower NaCl concentration (100 mM) has been observed faster development of stress reaction (anthocyanins accumulation and proline) but for higher NaCl concentration (200 mM) it was discovered after the second stage of treatment. The dose-dependent increasing of flavonol content has been observed for the wheat genotypes with more intense red-blue pigmentation after treatment with 150 mM and 200 mM NaCl concentration, respectively. The content of Na⁺ and K⁺ obtained at different levels of salinity on the basis of DW shown more than 3 times compared to the control significant increase of both ions under salt stress. The pigmented wheat genotypes which are characterized by higher anthocyanins content after salt stress treatment resulting in the significantly higher dry matter production.
First report about the identification of a partial sequence of FASCIATA in Thermopsis turcica, a Turkish endemic plant species with a polycarpellary gynoecium. Dilek Tekdal¹, Selim Çetiner¹.

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Thermopsis turcica is an endemic endangered plant species having a multi carpellate gynoecium with 2-4 functional pistils per flower. Although T. turcica is outstanding in terms of its carpel multiplication in the subfamily Papilionoideae of Fabaceae, to date no consideration has been given to the mechanism of its multi carpellary feature according to a literature review. Sizes of meristems usually are regulated by the interaction between gene WUSCHEL (WUS) and its negative regulators, such as genes CLAVATA (CLV) and FASCIATA (FAS). In the present study, identifying the regulatory mechanisms related to multi carpellary gynoecium in T. turcica was aimed. At the first stage of the present study, databases on whole-genome sequences of legumes were searched to find possible orthologs of FAS gene regulating floral meristem fate. Comparing these sequences, the degenerate primers were produced for amplification of putative orthologs from cDNA of T. turcica. After successful amplification, isolated genes were characterized via sequencing. Due to the sequence similarity, a verified 216 bp sequence was demonstrated as the FAS-like partial sequence. The putative gene was named TtFasc-1 and the partial CDS submitted to the NCBI database (accession number KT001128). This study may help to answer the roles of the identified orthologs in the regulation of floral structure found in T. turcica.
Drought is a major threat to agriculture and food production. Considering that over 70% of the globally available fresh water is used in agriculture to sustain crop production, it will be imperative to develop new crops with higher performance under water scarcity and able to consume less water and to maintain high efficiency. Functional genomics studies allowed a precise understanding of some of the physiological mechanisms governing plant adaptations to drought. Examples will be presented of how to improve crop yields under water scarcity through target-specific genetic engineering for modulation of stomata closure.

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With the aim to clarifying the role of Gibberellin (GA) / Hydrogen Peroxide (H2O2) signaling cascade in the regulating of the antioxidant capacity in the first internode of Triticum aestivum cv. Tir in response to deep sowing, we investigated the relationship between GA-mediated H2O2 production, the activiti-es of antioxidant enzymes in the first internode of Tir wheat. The results showed that the length of the first internode was increased in both H2O2 and GA-treated wheat seedlings at the depth of 10 cm. As seen by quantitative RT-PCR, H2O2 and GA up-regulated cell wall loosening-related genes (Glucanase EI and TaEXPB23) in the first internode of Tir under deep sowing condition (10 cm). Additionally, H2O2 and GA up-regulated also RBOHD and RBOHF genes (respiratory burst oxidase homolog genes) resulting in an increased in NOX (NADPH oxidase) activity. Furthermore, the activities of antioxidant enzymes (SOD, POX, APX, and GR) in the first internode of Tir increased the depth of 10 cm. The antioxidant enzyme activities were blocked by an inhibitor of GA under deep sowing condition. Moreover, pretreatment with NOX inhibitor also inhibited the activities of antioxidant enzymes in the first internode of Tir under deep sowing condition. These results suggest that GA-mediated H2O2 generation caused the induction of antioxidant defense systems in the first internode of Tir exposed to deep-sowing.
84 - Breeding of sharka disease in peach, nectarine and apricots.
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This study aimed to obtain resistant peach, nectarin and apricot genotypes to “Sharka” disease with
cross breeding method. Plum pox virus (PPV) causing Sharka disease is the most important viralagent
for stone fruits. This disease is most harmful to apricot, plum and peach trees. In this study, local apricot
varieties Hacıhaliloğlu and Kabaaşı, were crossed with foreign apricot varieties Stark Early Orange,
Rojo Pasion, Murciana and P 1908 (peach clone from Prunus davidiana) known to be resistant to PPV.
For peaches, commercial peach varieties Flored and Carolina were also crossed with Stark Early
Orange (apricot) and P 1908 clone (peach). A total of 365 genotypes from crossing among 12
combinations (FLxSEO, FLxP1908, CLxSEO, CLx1908, KAxSEO, KAxP1908, HHxSEO, HHxP1908,
MRxHH, MRxKA, RPxSEO, RPxAKA were tested with SSR markers ( P GS1.21, PGS1.24) and SSLP
marker (ZP002). Approximately 138 genotypes PPV resistance were expected to be candidate for PPV
resistance in future studies.
85 - Redox regulation under UV-B stress in Arabis alpina, a high altitude perennial relative of Arabidopsis thaliana.

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UV-B is an abiotic stress factor affecting photomorphogenesis, growth and development in plants. Reactive oxygen species (ROS) are by-products of normal metabolism in plants and can act as signal molecules at low quantities. On the other hand, excessive accumulation of ROS under stress conditions triggers oxidative stress and the counteracting antioxidant defence response. If oxidative stress could not be counteracted with the action of enzymatic and non-enzymatic antioxidants excess ROS can cause irreparable damage to DNA, lipids and proteins. Previous studies on UV-B tolerance have been carried out in Arabidopsis thaliana, which is not a UV-B tolerant species. In this work, we utilized Arabis alpina, a perennial plant that has been adapted to high altitudes, an environment more exposed to UV-B radiation for investigation of the role of antioxidant defence during plant response to high UV-B. For this aim, we used both A. thaliana and A. alpina to elucidate differential responses of these two plants to UV-B stress. ROS accumulation (amount of H2O2), lipid peroxidation levels, electrolyte leakage levels and maximum efficiency of PSII (Fv/Fm) were determined in both plants species following UV-B stress treatments. To elucidate the response of antioxidant defence, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GR) enzymes and NADPH oxidase (NOX) activities and isoenzymes were determined. In addition to this, changes in levels of plastid terminal oxidase, an alternative oxidase-like protein, was determined to elucidate differential responses of alternative electron sinks in chloroplasts of these two species under UV-B stress.
86 - Plant architecture measurement using a 3D reconstruction method suitable for high throughput plant phenotyping.

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In plant phenotyping, there is a demand for high-throughput, nondestructive systems that can accurately analyse various plant traits by measuring features such as plant volume, leaf area, and stem length. Existing vision-based systems either focus on speed using 2D imaging, which is consequently inaccurate, or on accuracy using time consuming 3D methods.

Objectives

• A new system combining both accuracy and fast three-dimensional (3D) reconstruction, suitable for high throughput plant phenotyping.
• Development of image processing methods for the identification and segmentation of plant organs (stem and leaf) from the 3D plant model.
• Evaluation of the accuracy of our system by comparing the measurements of our methods with ground-truth measurements obtained destructively.
Using lab models and on-line transpiration monitoring to select water efficient cultivars: a proof of principle in the banana bio(div)ersity collection.

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Water deficit is one of world’s major constraints in agriculture and will aggravate in the future. An important crop that needs vast amounts of water for optimal production is banana (Musa spp.). Millions of people depend on banana as staple food, while it is mostly grown in rain fed systems and hence very prone to fluctuating water availability. The International Transit Center of Bioversity International holds the world’s biggest collection of banana biodiversity (>1500 accessions).

Our aim is to evaluate the potential within this collection by phenotyping for climate smart agricultural usage. We have characterized the growth potential of 32 representatives of the Musa biodiversity under control and mild osmotic stress conditions. 5 cultivars were found growing relatively fast under control conditions with limited fall back under mild osmotic stress. To get more insight in the physiology, we gravimetrically monitored the individual plant transpiration in 10s intervals. We gain insight in the day to day, and within day, variations of transpiration rate. Banana is a crop originating from the humid tropics, and has developed a strong root pressure to ensure water transport in high humidity. We show that banana declines its transpiration rate near the end of the day, reducing transpiration water loss up to 30%. This feature is correlated to water use efficiency and is thus a valuable screening parameter for drought tolerance. We have selected 25 variables related to the transpiration pattern. Based on blind clustering within the 25 dimensional space we detect two major phenotypes in the Musa biodiversity: water consuming cultivars with a late transpiration decline, and cultivars with a more balanced water usage. Breaking up, and describing the daily transpiration proves useful as a cultivar selection tool, while also allowing insight in the plant physiology of transpiration.
Abiotic stresses such as cold, drought and salinity reveal profound impacts on plant proteomes. Plant exposure to stress induces a dynamic proteome response aimed at an establishment of a new homeostasis. The two-dimensional differential gel electrophoresis (2D-DIGE) analysis enables protein relative quantification leading to an identification of the protein spots revealing an enhanced abundance in stress-treated or stress-tolerant varieties which could be further tested as potential markers of stress tolerance. Proteomic experiments aimed at wheat, barley and melon proteome response to cold, drought and/or salinity were analyzed. The results of proteomic analyses were interpreted with respect to other physiological data such as parameters related to stress tolerance (membrane stability, LT50), water regime-related characteristics (water saturation deficit, osmotic potential), and others. The results reveal constitutively enhanced levels of several stress-related proteins (protective proteins, chaperones, ROS scavenging- and detoxification-related enzymes) in the tolerant genotypes with respect to the susceptible ones. Tolerant genotypes can efficiently adjust energy metabolism to enhanced needs during stress acclimation. Stress tolerance vs. stress susceptibility are relative terms which can reflect different stress-coping strategies depending on the given stress treatment.

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89 - Proteomic analysis of early stage infection in the tobacco-Tobacco mosaic virus (TMV) pathosystem upon topical application of dsRNA targeted to the TMV p126 gene. Naga Charan Konakalla¹,², Hema Masarapu², Andreas E. Voloudakis¹, Sebastien Carpentier³,4. ¹Laboratory of Plant Breeding and Biometry, Agricultural University of Athens, Athens 11855, Greece, ²Department of Virology, Sri Venkateswara University, Tirupati 517502, India, ³Department of Biosystems, KU Leuven, Leuven, Belgium, 4 - SYBIOMA: Facility for SYstems BIOlogy based MAss spectrometry, Leuven, Belgium. Presenting author: Andreas Voloudakis avoloud@aua.gr

RNA interference (RNAi) is a sequence specific, post-transcriptional gene silencing mechanism that is activated by double stranded RNA (dsRNA) in all eukaryotes. It is a conserved mechanism which protects the eukaryotic cells against viruses and transposons. Earlier, we showed that the exogenous application of dsRNA targeted to Tobacco mosaic virus (TMV) p126 gene, induced 65% resistance against TMV in tobacco. In the present study, we focused on the early stage changes at 15 minutes post inoculation in the tobacco-TMV pathosystem with and without exogenous application of dsRNA. TMV infection induces changes in the tobacco proteome as early as 15 minutes after inoculation, with the majority of the TMV-induced changes in the tobacco leaf proteome were not observed upon treatment of leaves with dsRNA p126+TMV. In this experiment, the topical application of dsRNAp126+TMV induced 66% resistance against TMV (7 dpi) in tobacco, which is consistent with our previous study. The identified differential TMV responsive proteins were found to be involved in photosynthesis, energy metabolism, stress and defense responses. We present the GO annotations of differentially-expressed proteins and determined the protein networks of important biological processes and molecular functions that are associated to TMV infection in tobacco employing Cytoscape software. To our knowledge, this study is the first high-throughput proteome analysis of TMV/tobacco interaction at very early stages of infection.
The essence of exploring and understanding mechanisms of plant adaptation to environmental stresses lies in the determination of patterns of the expression of proteins, identification of stress proteins and their association with the specific functions in metabolic pathways. To date, little information has been provided about the proteomic response of grapevine to the persistent influence of adverse environmental conditions. This article describes changes in the profile of protein accumulation in leaves of common grapevine (Vitis vinifera L.) seedlings in response to a prolonged effect of drought. Isolated proteins were separated by two-dimensional electrophoresis (2 DE), and the proteins whose level of accumulation changed significantly due to the applied stress factors were identified with tandem mass spectrometry MALDI TOF/TOF type. Analysis of the proteome of grapevine leaves led to the detection of many proteins whose synthesis changed in response to the applied stressor. Drought caused the most numerous changes in the accumulation of proteins associated with carbohydrate and energy metabolism, mostly connected with the pathways of glycolysis and photosystem II protein components. The biological function of the identified proteins has been discussed with reference to the stress of drought. Some of the identified proteins, especially the ones whose accumulation increased during drought stress, may be responsible for the adaptation of grapevine to drought.
This study aimed to identify genetic determinants of drought tolerance by using genome wide association study (GWAS) to identify proteins whose abundance variation can be related to the variation of ecophysiological traits. A panel of 251 maize genotypes was grown in the high-throughput phenotyping platform PhenoArch (Montpellier) under water deficit (WW) and well-watered (WD) conditions and in two replicates. At the pre-flowering stage, 1004 samples were taken on the last ligulated leaf and analyzed by shotgun proteomics. Ecophysiological traits were measured on the same plants. Protein identification and inference were performed using X!TandemPipeline (Langella et al., 2017, J. Proteome Res., 16:494-503). A total of 1950 proteins were quantified either by extracted ion current using MassChroQ (Valot et al., 2011, Proteomics 11:3572-77) or by peak counting. GWAS was performed for 3900 molecular phenotypes (=1950 proteins x 2 conditions) using FaST-LMM (Lippert et al., 2011, Nature Methods, 8:833-835). Most proteins showed significant abundance variations in response to water deficit and to genotype. Drought responsive proteins, like dehydrins, were highly induced under WD, while proteins of energy metabolism were down-regulated. PQLs (protein abundance QTLs) were detected for most proteins, and the strongest associations were detected for cis-PQLs, compared to trans-PQLs. Forty seven PQL/QTL colocalizations, involving 5 ecophysiological traits and 58 proteins, were identified. The results show the potential of high-throughput quantitative proteomics to decipher the determinisms of protein abundance regulation and to discover candidate genes and proteins potentially involved in the variation of plant phenotypic traits.
Insights into the root fermentative metabolism during growth in banana.
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Banana (Musa spp.), a non-model crop, ranks among the top ten staple foods, with a yearly production around 145 million tons (2014, FAOstat). Banana plants need vast amounts of water and even mild drought conditions are responsible of considerable yield losses. In a previous hydroponics experiment, we characterized root growth under control and osmotic stress via transcriptomics on three triploid genotypes that represent important subgroups of cultivated bananas (Zorrilla-Fontanesi et al., 2016). Based on large-scale mRNA sequencing, results pointed to an increased metabolic flow through fermentation and anaerobic respiration in fast growing tissues (root tips). To validate this outcome, we performed a greenhouse experiment. The root expression of 13 key genes and their paralogs involved in glycolysis, fermentation and (an)aerobic respiration (48 genes in total) was measured by RT-qPCR. Samples were clearly separated according to genomic group and treatment by using Partial Least Squares Discriminant Analysis (PLS-DA). Additionally, total activity of fermentation enzymes (pyruvate decarboxylase, lactate dehydrogenase and alcohol dehydrogenase) has been measured and compared to the expression levels/patterns of the corresponding paralogs in these gene families. Our results confirm the shift from a more aerobic to a more anaerobic metabolism to support root growth. However, they also show the complexity of dealing with polyploid genomes and gene families composed of paralogous copies with different expression levels/patterns in the same tissue. Linking expression data with total enzymatic activity helps to elucidate which pathways and which paralogous genes are ultimately affected by the applied treatment.